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STUDIES OF NEMATODES IN RELATION TO BROWN ROOT ROT OF TOBACCO IN ONTARIO[1]

BY W. B. MOUNTAIN[2]

Abstract

The root-lesion nematode, *Pratylenchus* spp. is proved to be the primary causal agent of the brown root rot disease of tobacco in Ontario. The root lesions resulting from nematode attack are undoubtedly further broken down by the activity of other soil organisms. At least two species of *Pratylenchus* are involved, differing significantly in pathogenicity and morphology. Nine other parasitic and potentially dangerous nematode genera also were found associated with tobacco roots in Ontario, but they occurred in very low numbers. The effect of the preceding crop upon brown root rot of tobacco was found to be related to nematode populations; and for eight preceding crops, significant effects upon the growth of tobacco occurred only in the presence of high populations of parasitic nematodes. Since *Pratylenchus minyus* Sher and Allen, 1953, is the more pathogenic species, the relation between this nematode and the tobacco host was studied in some detail. The optimum soil temperature for this nematode is at least 100° F. and it causes little stunting of tobacco below a soil temperature of 70° F. In a resistant variety of tobacco studied, an unusual mechanism of resistance upholds a host–parasite equilibrium wherein, despite high populations of parasitic nematodes within the roots, normal plant growth is maintained.

Introduction

After many years of intensive study, the large and very important field of the root rots still remains one of the most complex and least understood phases of phytopathological research. The reasons for this are clear when one considers the exceedingly complex microbiological equilibria in the soil environment. Any organism which may succeed in penetrating the root beyond its protective barriers is almost always followed by many other soil microorganisms and the resulting range of biotypes present in the wound often seems limited only by the extent of necrotic tissues available at that particular time. With such a complex, the difficulties lie in proving which organism was responsible for the initial penetration and in attempting to evaluate the possible effects of the other components present.

Organisms which very frequently seem to be associated with root rot diseases are the nematodes. Single root lesions often will contain hundreds of such

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Joint contribution from the Department of Botany, University of Toronto, Toronto, Ontario, and the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario—Contribution No. 1405.

Based on a thesis presented in April, 1953, to the University of Toronto in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] *At the time graduate student, Department of Botany, University of Toronto; now Associate Plant Pathologist, Science Service Laboratory, Harrow, Ontario.*

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animals. Yet, for many years, the presence of the nematodes was largely ignored by plant pathologists or at most they were regarded as rather innocuous associates, of unknown but presumably small importance, of any fungal or bacterial components present in the lesion.

It has been realized for some time that a critical study of the role of nematodes is essential in all types of root degeneration complexes. No one has shown conclusively that the nematodes concerned with these diseases are capable, under aseptic conditions, of acting as primary root invaders, or exactly what amount of root damage will result from an attack of these organisms, when the normal microfloral elements are prevented from complicating the lesions. The separation of plant parasitic nematodes from their microfloral associates proved to be a difficult task and, in the study of the parasitic relations of the nematodes, the maintenance of such a separation for an extended period of time seemed at first to be an almost unattainable objective.

For the present studies, the brown root rot disease of tobacco was selected because it had been shown previously that there is a basic association between the disease and the root lesion nematodes, *Pratylenchus* spp. Fungi and bacteria appear to have no consistent effect.

The objectives of the present research were twofold: (a) a detailed study of the disease as it occurs in Ontario and (b) an attempt to devise new approaches and techniques which might tend to bring present day nematode research in line with the more critical approaches used in other phytopathological problems.

Literature Review

Brown root rot of tobacco was first described and named in 1926 (20) and at that time had been under surveillance for approximately ten years. It was reported from Ontario in 1935 (21). The search for a fungus responsible for the disease ended in failure. *Fusarium* spp. occurred frequently (11, 17, 19, 20). *Rhizoctonia* sp. and *Trichoderma* sp. had also been reported (11, 18, 27). However, in 1948, Jenkens (16) reported that either isolations from young lesions were negative or the isolates were so variable and inconsistent as to preclude the possibility of a primary bacterial or fungus parasite.

This failure to isolate a disease-producing organism, coupled with the observed effects of certain preceding crops upon the disease, led many workers to investigate the possibility of substances toxic to tobacco arising as a result of a preceding crop, especially timothy and corn (1, 4, 5, 6, 7, 26, 27, 35, 36).

Stover in 1951 (34) showed that varieties both resistant and susceptible to brown root rot responded similarly to a root rot induced by the addition to the soil of decomposing crop residues and this similarity in response suggested that the stunting and browning resulting from excess crop residues were something different from the brown root rot disease.

The first report to associate nematodes with brown root rot of tobacco was in 1931 by Lehman (24). In that same year, Steiner (31) examined the roots of tobacco submitted by Lehman from North Carolina and observed *Tylenchus pratensis*, *Aphelenchus avenae*, and *Cephalobus* sp. The symptoms described

by Lehman are identical with those originally described in 1926 for brown root rot. Reports of these nematodes on tobacco in other localities did not appear for several years. In 1935 Koch (21) reported that nematodes of a type distinct from *Heterodera* were frequently observed in lesions on tobacco roots in Ontario. The following year, Hildebrand and Koch (13) stressed the potential importance of the meadow nematode in any root rot complex.

Nematodes on tobacco were reported from South Carolina in 1942 (10), in Virginia in 1944 (15), and in Kentucky in 1947 (38, 40). In 1951, Graham (11) reported that the brown root rot occurring in South Carolina is caused by two species of *Pratylenchus*, *P. zeae*, and *P. leiocephalus*.

Symptomatology

Brown root rot of tobacco, as its name implies, is recognized by the presence of brownish lesions on the root system of the affected plant. These lesions usually begin as small, water-soaked areas on the surface of branch roots, and the affected areas gradually expand and turn brown. In advanced stages, the lesion, complicated by secondary organisms, girdles the root; the epidermis and cortex break down and slough off, leaving only the stele in the affected area and often giving the root a beaded appearance (Fig. 9). As the disease progresses, the lateral roots are killed, and a stimulation to produce new secondary roots from the stem above the affected areas results in a very shallow root system. In severe cases, this root system is reduced to a few necrotic roots lacking root hairs and with very few branch roots (Fig. 3, 4).

Such a root breakdown is reflected in the appearance of the aboveground parts in symptoms typical of disturbed water and mineral intake which consequently are not specifically diagnostic of this disease. Stunting is the first and frequently the only aboveground symptom appearing in the field (Fig. 3), with patches of stunted plants occurring in rather discrete areas within the affected field (Fig. 8). With increasing severity of the disease, especially at high temperatures, the progress of the disease is as follows: extreme stunting, or even complete cessation of growth; reversible wilting of the lower leaf blades under optimal transpiration conditions; reversible wilting of the leaf petioles; chlorosis, beginning with mottling of the oldest leaves and continuing until the entire blade is yellow; chlorosis extending to all but the youngest leaves; irreversible wilting and leaf collapse beginning with the oldest leaves; and occasionally the death of the plant (Figs. 5, 6). Usually the irreversible wilting stage is reached only in high temperature experiments.

Materials and Methods

Extraction of the Nematodes

In order to determine relative nematode infestations, the Baermann funnel technique has been used successfully. This technique is fully described by Filipjev and Schuurmans Stekhoven (8) and makes use of the fact that when infested roots are immersed in water, the nematodes will leave the roots and settle to the bottom of the container. For this work reported here, a bank of

ten 8-in. glass funnels supported in a wooden rack was used. Cones of plastic fly screen fitted inside the funnels were found to be very effective in supporting the roots. The populations of plant parasitic nematodes emerging from the roots were calculated on the basis of numbers per gram of dry root tissue. This technique will not give the total nematode content of roots, but was found to be very effective in indicating relative differences between samples tested.

Many plant parasitic nematodes never enter the roots, but act as external parasites feeding upon root hairs and the root tissue. When the soil is washed from the roots, as in the method described above, those forms are lost and hence their presence may never even be suspected. In order to extract these nematodes, it is necessary to screen the soil surrounding the roots. For this purpose, the soil screening technique as described by Cobb (3) was used. Three copper screens of 35 mesh, 100 mesh, and 200 mesh were used. The residues from each screen are collected and examined for nematodes.

Staining Techniques

Single nematodes are best handled with a bamboo pick sharpened to a very fine point. With practice, nematodes can be transferred rapidly under the dissecting microscope using this splint. For taxonomic studies, nematodes are transferred from the watch glass to a small quantity of water in a 30 ml. beaker and an equal quantity of boiling water slowly added to the beaker. The killing of nematodes in this manner relaxes the muscles and prevents distortion. The nematodes are then transferred to warm formal – acetic acid fixer or 5% formalin, fixed for at least 24 hr. or stored in these solutions indefinitely.

For much of the study Goodey's rapid method of clearing and staining with hot lactophenol tinted with cotton blue was used (9). This clears and stains nematodes quite well. A slower clearing method which results in less distortion consists of placing the nematodes, after killing and fixing, in a 1.5% glycerine solution and then allowing the water to evaporate over a period of at least four weeks. No staining is necessary. Living nematodes were occasionally placed directly in Fleming's strong fixer. Occasionally *en face* examinations were made following Buhrer's method (2).

To demonstrate nematode invasion of roots, Graham's method of staining with Fleming's weaker solution was very effective (11). Goodey's lactophenol and acid fuchsin was also used frequently and very good results were obtained using the modification of Goodey's method developed by McBeth *et al.* (25).

Assessing Disease Severity

The disease incidence was assessed on the basis of the net increase in green weight of the entire tobacco plant. Often the only consistent plant response to nematode attack is a general stunting of the plant and it was felt that the weight increase is the most accurate and reproducible measurement of this retarded growth. Preliminary experiments indicated a high degree of correlation between the number of parasitic nematodes per gram of root and the net increase in the green weight, under otherwise similar environment.

The Use of a Selective Soil Fumigant in the Experimental Procedure

The normal and most desirable method of assessing the pathogenicity of a particular organism involves a comparison of the host response to the same environment in the presence and in the absence of the pathogen in question. With soil-borne pathogens, usually a pure-line culture of the organism is added to sterilized soil and this treatment is compared with controls in which no inoculum is added to sterilized soil. An objection to this technique is that the pathogenic organism is operating in an artificial medium in that there is little or no chance for the expression of normal associative effects with other microorganisms. It would be better to avoid the use of sterile soil and deal with samples essentially identical except that in one case the organism would be present and in the other absent. However this technique is always difficult to achieve. A modification of this latter technique was used in the experimental work reported herein.

A method was devised whereby the nematode population may be controlled without seriously disturbing the microfloral population. A soil fumigant, ethylene dibromide, was selected, which, although an effective nematicide, is reported to be a poor fungicide or bactericide (41, 30). Thus, if one portion of a soil sample is treated with a minimal concentration of this fumigant and another portion left untreated, any beneficial effects resulting from growing plants in the treated soil are likely to be due primarily to the reduction in the nematode population. The concentration of fumigant used throughout the experimental work reported herein was 0.2 ml. of a commercial formulation of 83% (by weight) of ethylene dibromide, per 1000 gm. of soil.

Aseptic Culturing of Parasitic Nematodes

One weakness of much of the research dealing with nematode diseases has been the difficulty of proving by recognized pathological methods whether or not a disease is induced by the nematode alone. From the very beginning of this investigation, it was felt that techniques must be evolved so that the rules of proof of pathogenicity might be applied and the true role of the nematode in this and other root rot diseases might be elucidated. The first step in this procedure would be to obtain cultures of the plant parasitic nematode in question, free from bacterial and fungal contaminants. Three methods of culturing nematodes were attempted during this investigation.

The first method was the culturing of the nematode directly upon an agar medium. Many different media were tried, including tobacco and corn root extracts, with negative results. To date no plant parasitic nematode has been cultured by this method.

A second method used in attempting to culture nematodes involved the growing of a suitable host plant in vermiculite using a nutrient solution and then building up nematode infestations upon the roots (34). This technique certainly is a step forward as it will show the effect of nematode feeding in the absence of many soil microorganisms as compared with the effect in the microbiological complex of the soil environment. However, as the amount of

necrotic root tissue increases, so does the complexity of the microbiological association and indeed, after a relatively short period of time, one is left with a system, much simpler perhaps than the soil microbiological complex, but nevertheless with more components than desired. The method is cumbersome and contamination cannot be prevented. It is very unlikely that a pure culture could ever be established by this method. It has also been found so difficult to establish such nematode cultures that mass inoculations involving nematodes of all types have been the only successful experimental method applied thus far.

The third approach investigated in this study was rearing the plant parasitic nematodes under aseptic conditions on root tissue culture. An original technique which achieves this objective was evolved and is described in detail elsewhere (28). It is felt that this method will be broadly applicable in future nematode disease investigations. It may be briefly described as follows. Root tissue cultures are grown in petri dishes using White's tissue culture medium (42) without modification, except that the solution is made up into a 0.75% agar medium. When the root culture has become established, plant parasitic nematodes, after being surface sterilized by washing in sterile water and in 0.1% streptomycin, are placed beside the root culture. The nematodes enter the root, feed, and eventually reproduce. With the petri dish inverted, the culture can be studied in detail under the low powers of a compound microscope. In all, the roots of three host plants, corn, tobacco, and red clover, have been used in this manner; and two species of *Pratylenchus*, both of which will cause brown root rot of tobacco, were cultured. Cultures were maintained for periods up to four months; the original inoculum increased over a thousand times.

Nematodes Associated with Brown Root Rot of Tobacco in Ontario

Detailed taxonomic studies were carried out upon nematodes within and upon diseased tobacco roots obtained from tobacco fields throughout southern Ontario. Hundreds of nematodes were thus examined and in Table I is shown the identification and occurrence of over 1500 individuals. For the identification, each specimen was cleared and studied under the oil immersion lens of a compound microscope. Table I gives a general picture of the complex involved in the disease, but as will be seen later, this complex can be altered by certain factors.

It may be seen from Table I that over 40% of the nematodes associated with the disease are known plant parasites. A further 14% are also spear-bearing nematodes, some of which, at least, are parasitic.

The most commonly occurring parasitic nematode is the root lesion nematode, *Pratylenchus* spp. Surveys have indicated this nematode to be widespread throughout the tobacco growing areas of Ontario. Formerly most of the forms occurring in North America were believed to be *Pratylenchus pratensis*, but following Thorne's redescription of this species in 1949 (37), it was found

TABLE I

NEMATODES FOUND ASSOCIATED WITH ROOTS OF TOBACCO PLANTS AFFECTED BY BROWN ROOT ROT OF TOBACCO IN ONTARIO

Host relationship	Genus and species	Number encountered	Relative frequency, %
Known plant parasites	*Pratylenchus* spp.	575	38.3
	Tylenchorhynchus claytoni	9	0.6
	Hoplolaimus coronatus	6	0.4
	Tylenchus filiformis	6	0.4
	Ditylenchus sp.	2	0.13
	Boleodorus sp.	2	0.13
	Xiphinema americanum	2	0.13
	Telylenchus sp.	1	0.07
	Rotylenchus sp.	1	0.07
Other stylet-bearing nematodes	*Aphelenchus avenae*	115	7.7
	Aphelenchoides spp.	12	0.8
	Longidorus spp.	2	0.13
	Dorylaimus spp. (and other Dorylaims)	87	5.8
Saprophytic associates	Including: *Rhabditis* spp. *Cephalobus* sp. *Eucephalobus* sp. *Diploscapter* spp. *Acroboles complexus* etc.	679	45.76

that most of these forms were not of that species. Sher and Allen (29), in revising the genus in 1953, have described 10 closely related species. Several other species have since been discovered, which are not yet described. At least three species of *Pratylenchus* occurring in Ontario will cause brown root rot of tobacco; two of these were investigated in some detail in this study and their pathogenic capabilities will be assessed in this paper.

Tylenchorhynchus claytoni Steiner, 1933, is far more common in Ontario soils than Table I would indicate. However, it was rarely found associated with tobacco. *Tylenchorhynchus claytoni* frequently occurs in large numbers on the roots of wheat and oats in southwestern Ontario. Steiner (23) reports, "This is a form that is continuously observed on dwarfed plants. We suspect this species to be very poisonous to the host plant".

It is not certain whether *Hoplolaimus coronatus* Cobb, 1923, has been observed on tobacco prior to this study. Steiner notes that they somewhat resemble the meadow nematode in their life cycle and they may function both as ectoparasites and endoparasites. "Since they are large and powerful migratory types that move in and out of a root, their destructive action should not be underestimated" (33).

Xiphinema americanum Cobb, 1913, is common in the lighter soils of southwestern Ontario. It is also widely distributed in the United States and attacks a wide variety of crops. "*Xiphinema* is an ectoparasite, which, with its long, needle-shaped stylet, feeds on the vascular tissues from the root surface" (33).

The other parasites isolated from tobacco roots, *Ditylenchus* sp., *Tylenchus filiformis*, *Boleodorus* sp., *Tetylenchus* sp., *Rotylenchus* sp., are quite rare in southwestern Ontario soils. They are all believed to be parasitic and quite destructive.

The nematodes *Aphelenchus*, *Aphelenchoides*, *Dorylaimus*, and *Longidorus* are usually found associated with known parasitic nematodes and it is assumed generally that they may function as parasites only under certain conditions. A few species of *Aphelenchoides* are obligate plant parasites; other forms are predaceous or saprophytic, but most are suspected of at least a limited amount of plant parasitism. Steiner regards *Aphelenchus avenae* Bastian, 1865, as a facultative plant parasite with perhaps dominant necrobiotic tendencies. His observations indicate that this is not a harmless form (32). Many species of *Dorylaimus* are believed to be predators, but others are perhaps vegetation feeders; however, the bionomics of both *Dorylaimus* and *Longidorus* have not been elucidated.

The nematode complex associated with brown root rot of tobacco may, in many cases, be modified to a very great extent by certain factors. These have been found to include soil temperature, soil type, crop rotation, varietal resistance, and geographic location. The modifications which result from many of these factors will be discussed in detail in this paper.

Experimental

Studies on Penetration and Subsequent Colonization

The following studies were made on root tissue cultures of corn and tobacco inoculated with the root lesion nematode, *Pratylenchus minyus* Sher and Allen, 1953. Since it is upon corn that *Pratylenchus minyus* builds up, this host was used to maintain the pure cultures and for certain studies of host–parasite relations. The observations on tobacco were somewhat detailed for two reasons: first, that it is the interaction between this nematode and tobacco which gives rise to the brown root rot disease and, secondly, that the tobacco root in culture is usually more transparent than the corn root and detailed observations subsequent to nematode penetration can be carried out.

The tissue cultures were set up following the technique outlined previously (28). When the nematode colonies in the corn root culture had become established, new corn roots or tobacco roots were placed beside these original cultures, until they too had become infected and were then transferred to separate petri dishes as subcultures.

Corn Root Observations

When a fresh corn root was added to a well established nematode colony, many nematodes were almost immediately attracted to the root and soon were clustering about it. It was interesting to note that the adult and late juvenile stages were most frequently attracted to the root. The root tip region attracted more nematodes than any other portion and they seemed to feed upon the cells of that region, both the meristematic cells of the root tip and the larger

cells of the root cap. Because of the compactness of the corn root, it was not often possible to observe the nematodes within the tissue. In the case of tobacco, however, such observations could be made. Occasionally, nematodes could be observed within the finer lateral roots of corn. Although nematodes frequently were seen partly buried in the tissue (Fig. 15, 16), the complete process of penetration could not be followed. Penetration seldom appeared to take place at the growing point, as stained roots very seldom showed nematodes in this region. Hastings, studying infection of oat seedlings by *Pratylenchus pratensis* seldom found nematodes near the root tip (12).

After a fresh corn root had been placed beside a very active older culture and been penetrated by a large number of nematodes, there was an increase in the formation of lateral roots, so that within 10 to 14 days, such a newly-infested culture would form a much-branched root system, whereas a non-infected culture of the same age produced very few lateral roots. Wounding of the root by puncturing with a needle often had the same effect. When these newly-emerging lateral roots were attacked by a large number of nematodes growth soon ceased and the length of the lateral root was fairly indicative of the severity of attack. Once again, although the root tips were heavily attacked externally, penetration of the branch roots did not occur in this area. The region where penetration occurred was back in the more mature areas of the lateral roots and these showed up as lighter regions, which, upon microscopic examination, were seen to consist of disrupted epidermal cells (Fig. 17). Occasionally the host responded with cellular hypertrophy in the region of these lighter areas (Fig. 12), although this response was never observed in the larger main roots. One such lateral root, which was stained 48 hr. after the initial nematode attack, contained over 100 nematodes largely in pockets, and it is believed that at least several must have entered each single break in the epidermis (Fig. 11). Living nematodes were observed within the smaller lateral roots and appeared to have made their way through the tissues from the main root into the newly-developing lateral roots (Fig. 14). Within the finer lateral roots, they frequently were stretched out at full length parallel to the conducting tissue, and displayed only a limited amount of movement. Although, in dicotyledons, it is generally believed nematodes feed upon the cambial cells, they were observed here feeding upon cortical cells quite some distance from the vascular tissue and even upon the undersurface of the epidermal cells.

After a period of about four weeks, the root cultures began to show brownish areas similar to those observed upon corn roots in the field. These lesions on the cultured roots were devoid of microorganisms other than the nematodes. There was very little disruption of tissue; the epidermis remained intact, except where the original penetration occurred, and the epidermal cells appeared to be living. Most of the necrosis was confined to the cortex. In a very few cases there was extended tissue disruption. One lateral root was observed in which the epidermis and much of the cortex had been torn apart and the disruption extended to the stele.

Tobacco Root Observations

Inasmuch as tobacco roots grew more slowly than those of corn, they usually were cultured for a week or longer prior to being transferred to a plate containing nematode infested corn roots. Cultured tobacco roots seldom formed lateral branches, but they produced abundant root hairs. In these roots three regions could readily be recognized: the root tip, a narrow area producing many root hairs, and a much thicker mature region. The nematodes were almost immediately attracted to the tobacco roots and within a period of two to three hours might travel through the agar a distance of one to two centimeters from the old culture. The adults and late juveniles were once again the migratory stages of the nematode and immediately attacked the root along its length, but more especially at the older mature region and at the growing point. They appeared to feed externally upon the meristematic cells in the growing region and caused growth of the root to cease within a day or two. As in corn, no nematodes have yet been observed to enter the tobacco root at the growing-point region, and within two to three days, they frequently moved away from this area and paid no more attention to it. They also attacked the root-hair region and very frequently penetrated the epidermis and partially entered the cortex. The root is very narrow in this region, and evidently the cortical area was not large enough for the nematodes soon left this part of the root. The mature region, where the root is quite thick, was the locus of greatest attack. Upon reaching this region, the nematodes began to feed almost immediately upon the epidermal cells. Frequently, they would lie along the root surface in a serpentine position and, once feeding commenced, they became relatively quiescent. The actual penetration of the cell wall by the stylet was not observed, but the basal knobs could be seen pulsating back and forth, a phenomenon associated with feeding. Frequently, in as short a time as four to six hours, the adult or late juvenile nematodes had pushed aside the epidermal cells and partly invaded the cortex to about a third of their body length. They often remained in this position for several hours and could be observed feeding upon cortical cells. However, they usually continued their invasion of the cortex and frequently within 8 to 10 hours were completely inside the root tissues. After this they became relatively immobile. The mouth changed position frequently as the nematode fed upon new cells, but the rest of the body did not change position readily. Eggs were laid almost immediately after penetration and hatched within seven to nine days.

After four to six days the nematodes within the root began to move about quite readily, and about this same time the cortical tissue began to break down. The tissues in the invaded area turned brown and appeared to be killed. In one culture, which was kept free of contaminants for over a month, all of the nematodes in the lesion had died within that time. The cortical cells surrounding the nematodes were killed and disrupted and it is assumed that the nematodes died of starvation. The epidermal cells also appeared to be necrotic, but retained their original shape and did not disintegrate. The

cells of the rest of the root appeared to be alive. It thus appears that under aseptic conditions the lesion did not approach the stage of advancement observed in the soil. This suggests that subsequent breakdown may be correlated with the activity of various secondary organisms.

Nematodes apparently enter tobacco roots in one of two ways. (1) In the means of entry perhaps most commonly observed in culture, the epidermal cells were forced aside without noticeable injury but between them was left a long narrow gap that remained open for a considerable period after the nematode had entered the root. (2) In the second type, the opening appeared to have been created by the complete collapse of an epidermal cell, and a clear space, exactly conforming to the former cell limits, was thus formed (Fig. 13). No evidence of the collapsed epidermal cell could be found. It is assumed that the nematode, in pushing its way into the opening, carried the cell remnants into the cortical region. In the case of either method, a large and apparently permanent opening through the epidermal barrier was thus available as an infection court for secondary microorganisms. Thus far, no evidence of hypertrophy has been observed in these roots. Tobacco roots appear to be more rapidly broken down by nematode activity than corn roots. This tendency also exists in the field.

Life Cycle of Pratylenchus minyus

The complete life cycle has never been elucidated, but some observations are recorded here. Eggs laid in the agar outside the root hatched in a period of six to seven days. In one case, out of 25 eggs, 15 hatched on the sixth day, nine on the seventh, and one did not develop. Hastings, working with the bisexual *Pratylenchus pratensis* attacking oats, reported that eggs hatched under 12 days (12). Graham suggested that for *Pratylenchus zeae* (11) the period required for hatching was 15 to 20 days.

The first juvenile stage of *Pratylenchus minyus* is very weak and in no case in which roots were inoculated by surface-sterilized eggs did penetration by newly hatched juveniles occur. This observation apparently does not agree with those of Hastings who reported all stages capable of entering roots. It is interesting to recall that he worked with a bisexual form, whereas the form used here is monosexual and presumably parthenogenetic. Although loss of infectivity by the first juveniles may reflect species differentiation, it is possible that part of the mechanism for greater activity of the bisexual form results from hybrid vigor. The most infective stage of this species appeared to be the preadult juvenile form, followed closely by the adult itself.

The time required for completion of the life cycle was not ascertained, but the time from initial penetration to the emergence of nematodes of all stages was about 28 days. Usually more adult forms emerged from the roots than had been supplied in the original inoculum, and this observation suggested that the entire life cycle had been completed within this time. Graham believed the period from egg to adult to be 35 to 40 days for *Pratylenchus zeae*. Hastings found that the life cycle of *Pratylenchus pratensis* was completed within 54 to 65 days.

The Effect of Previous Crops upon Brown Root Rot and the Associated Nematode Complex

One of the earliest observations made in the study of this disease was the effect of previous crops upon the severity of brown root rot of tobacco. Johnson *et al.* (20) reported the tobacco crop to be poorest when grown after timothy, and to be only slightly less poor when grown after corn and after clover. The best yield appeared to be obtained when tobacco followed tobacco. In Ontario, Koch observed that the factor most consistently influencing severity was the preceding crop (22). He showed that when one or more crops of corn immediately preceded tobacco in rotation, the disease was always severe. Soybean and timothy also exerted a similar but considerably less pronounced influence.

It was decided to test soils in which various crops had been growing in an attempt to evaluate their effects upon the tobacco plant and to study the nematode complex associated with such rotations, with particular emphasis on the meadow nematode populations. Altogether, soils have been tested in which had been growing eight different crops which were either normally associated in the tobacco rotation or ones in which meadow nematode populations were known or suspected to be high.

Samples were selected of Harrow Sandy Loam in which the six different crops of timothy, corn, tobacco, red clover, soybean, and rye had been growing for at least one season. Subsequently, experiments involving tobacco in strawberry and peach soil were carried out, since in both of these soils root degeneration problems, involving parasitic nematodes to an undetermined extent, exist in Ontario. It was desired to know whether there might be some common etiological basis for these three root diseases and so it was decided to include these in the test.

The soils in question were brought into the greenhouse in the summer of 1951 and placed in divided greenhouse flats previously sterilized by steam. The specific crop was grown in each soil continuously for one year in order to ensure that each of these individual soils had as high a nematode population as the particular crop would support. The timothy soil had been growing timothy for about 50 years, ever since the land had been under cultivation. In the tobacco soil, there had been very severe brown root rot the preceding summer. The peach soil was from a former peach orchard where several attempts to re-establish the orchard had failed owing to death of the young trees. The strawberry soil had been growing strawberries for over five years. The previous history of the other soils was not known.

In the spring of 1952, the plants in all flats were cut off at the soil level and the aerial parts discarded. The soil and roots in each flat were mixed thoroughly, and one half of each flat was fumigated with 83% ethylene dibromide at the rate of 0.2 ml. per 1000 gm. of soil. The other half of each flat was left untreated.

Ten days after fumigation three previously weighed tobacco seedlings were planted in each half-flat of soil and the entire series was placed at random

on a greenhouse bench. After six weeks, each plant was removed and the soil carefully washed away from the roots. The disease index was assessed on the net increase in green weight of the entire plant. The nematodes were extracted by the Baermann funnel method and parasitic populations calculated on the basis of nematodes per gram of dry root. Differences in growth between treatments was assessed using "Student's" *t*-test. The resultant effect of six preceding crops on the growth of tobacco and the populations of *Pratylenchus* appear in Table II; the relative growth of tobacco in the various soils is shown in Fig. 1.

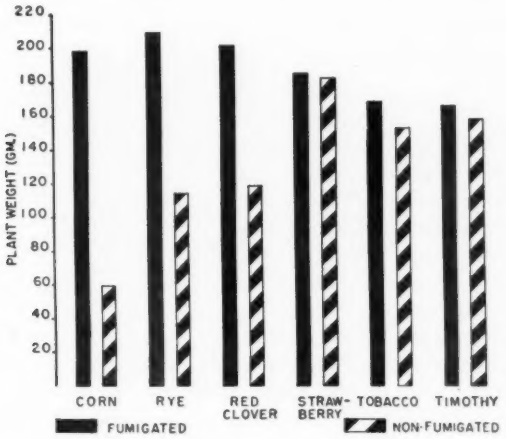


FIG. 1. The effect of preceding crops upon brown root rot of tobacco as assessed by the increase in green weight, in both fumigated and nonfumigated soils.

TABLE II

EFFECT OF PREVIOUS CROPS UPON GROWTH OF TOBACCO AND ROOT LESION NEMATODES

Preceding crop	Soil treatment	Net increase in green weight (gm.)	*Pratylenchus* per gram of root
Timothy	Fumigated	166.5	0
	Nonfumigated	158.0	0
Tobacco	Fumigated	168.8	0
	Nonfumigated	154.1	2
Corn	Fumigated	196.0	5
	Nonfumigated	58.7†	215
Red clover	Fumigated	202.9	10
	Nonfumigated	118.7*	307
Rye	Fumigated	209.4	34
	Nonfumigated	115.4*	163
Strawberry	Fumigated	184.7	0
	Nonfumigated	183.1	1
Soybean	Fumigated	—	122
	Nonfumigated	—	356

† *Significant at 0.01.*
* *Significant at 0.05.*

Tobacco after Timothy

The increase in green weight following soil fumigation was not significant. The fact that timothy sod has long been suspected to be a precursor of severe brown root rot does not appear to be supported by these results. No parasitic nematodes were removed from the roots of tobacco growing in this soil. Hildebrand, however, found that timothy roots could become heavily infested with *Pratylenchus* sp. (14). Thus, one might have expected to find brown root rot of tobacco following timothy if populations of root-lesion nematodes such as Hildebrand found had occurred here.

Tobacco after Tobacco

As mentioned earlier, tobacco grown two years prior to this experiment in the same soil showed severe brown root rot and extreme stunting. At that time, nematode studies indicated that an enormous population of *Pratylenchus* was present. After approximately sixteen months of continuous growing of tobacco, during which time no roots, and consequently no nematodes, were removed from the soil, the nematode population, as indicated by the results of this experiment, appeared to be very low. The increase in green weight of the plants due to soil fumigation was not significant, and therefore the disease had disappeared. Practically no parasitic nematodes could be found except for a very few specimens of *Pratylenchus* and a trace of *Aphelenchus avenae* and *Aphelenchoides* spp. Thus, earlier observations of the disappearance of brown root rot following continuous cropping of tobacco is confirmed. This marked decline in both nematode population and disease, in a period of less than two years under favorable environmental conditions for both the nematode and the host, must indicate that the pathogen is incapable of maintaining itself permanently upon this host.

Tobacco after Corn

Soil fumigation resulted in a very significant increase in growth. Correlated with this was a 95% decrease in the parasitic nematode population and thus this lowering of the nematode population was once again correlated with the elimination of the disease. A study of the nematode complex within the roots of tobacco following corn showed that approximately 30% of all the nematodes occurring consisted of a single species of *Pratylenchus*. This has been identified as *Pratylenchus minyus*, Sher and Allen, 1953. Although males were described by Sher and Allen (29), the species, as it occurs in Ontario, is monosexual, having been reared on root cultures for many generations without the appearance of a single male.

Tobacco after Rye

The increase in growth as a result of soil fumigation was significant and was correlated with an 80% reduction in the *Pratylenchus* population. Two species were found to be present, about 75% of which were *Pratylenchus minyus* and the remainder *Pratylenchus penetrans* Cobb. A rye–tobacco rotation is the one most frequently used in the tobacco producing areas of

southwestern Ontario. However, under field conditions, brown root rot is rarely serious when this rotation is followed. The reason for this fact is believed to be that a high inoculum potential cannot be attained unless rye is grown continuously for several years. In view of the fact that the same species of *Pratylenchus* builds up both on rye and corn, it would appear that these two crops should never be used together in any rotation involving tobacco.

Tobacco after Red Clover

There was a significant increase in plant weight as a result of soil fumigation. Although there were approximately 92 more *Pratylenchus* per gram of tobacco root in this rotation than there were in tobacco after corn, there was much less stunting of the plants in the red clover than in the corn series. The *Pratylenchus* involved in the red clover series, however, is not *Pratylenchus minyus*. Although it closely resembles *Pratylenchus scribneri* Steiner, it differs from this species in two important characters: (*a*) the vulva is over 80%, (*b*) males are extremely common, occurring with females in the ratio of 1 : 1. It is considered to be a separate and undescribed species. It may be assumed that this clover-*Pratylenchus* penetrates and colonizes the tobacco roots as does *Pratylenchus minyus*, although detailed culture studies have not been carried out. The reason for the distinctly lower pathogenicity of this species has not yet been elucidated.

Tobacco after Strawberry

Correlated with the lack of growth response as a result of soil fumigation was the fact that there were almost no nematodes present in the roots. Hildebrand found *Pratylenchus* sp. to occur in strawberry roots only when strawberry followed timothy in rotation (14).

Tobacco after Soybean

The growth data from this series were not included in Table II because an outbreak of blue mold occurred on plants in both treated and nontreated soils. There was, however, an average of 346 *Pratylenchus* per gram of tobacco root in the nonfumigated plots. This was a mixed population consisting of *Pratylenchus minyus* and the clover-*Pratylenchus*. The effects of soybean rotation upon tobacco will be further studied as soon as possible.

Tobacco after Peach

A serious problem at present under investigation at the Harrow Laboratory is the difficulty of reestablishing peach on the sites of old peach orchards in southwestern Ontario. Results, soon to be published, indicate that the stunting and killing of these peach seedlings is associated with the presence of large populations of parasitic nematodes, including the root-lesion nematode, *Pratylenchus penetrans* Cobb. When tobacco was planted in such nematode-infested peach soil, the plants were severely stunted and large numbers, exceeding 500 per gm. of tobacco root, of *P. penetrans* were found infesting the roots of the affected plants. The relationship of *Pratylenchus penetrans* to brown root rot of tobacco was not further investigated in this present study.

The differences in the growth of tobacco in the various fumigated soils were not found to be significant, and, therefore, there does not appear to be any great effect of the preceding crops upon the growth of tobacco in the absence of parasitic nematodes. Of those tested, corn, red clover, soybean, rye, and peach soils appeared to be the only ones supporting high populations of the root lesion nematode and these were also the only ones having a depressing effect upon the growth of a subsequent tobacco crop.

The Effect of Soil Temperature upon Brown Root Rot and the Parasitic Nematode Population

Crop damage caused by nematodes is generally considered to become more severe the farther south one proceeds in the United States and this is interpreted as a temperature effect. If this be true, the same trend should exist with respect to brown root rot, but unfortunately very little experimental work involving the effect of soil temperature upon this disease has been carried out. Jenkens (16) reported that a temperature of 75° to 80° F. enhances root rotting and accelerates the reproduction rate of the nematode. Graham (11) reports root rot as slight to moderate at 60° to 70° F., moderate to severe at 70° to 80° F., and severe at 90° F., while the increase in the *Pratylenchus zeae* population was in the proportion of 6 : 23 : 45 at the temperatures indicated. It was decided to study the effect of soil temperatures using soil infested with *Pratylenchus minyus*, in order to assess critically the effect of soil temperature upon plant growth and the causal nematode.

The soil used was naturally-infested Harrow sandy loam, obtained at the Harrow laboratory from corn plots in which this crop had been grown two years in succession. The corn roots were chopped fine and mixed thoroughly with the soil. Six Wisconsin soil temperature tanks were used in which the soil temperatures ranged from 50° to 100° F. in 10-degree increments. The soil was placed in steam-sterilized, 7-in. pots each of which was suspended in a large Wisconsin can so that the soil surface was slightly below the top of the can, and the space between the two was packed with moist sand. There were eight pots for each temperature. Four pots in each tank were fumigated with ethylene dibromide at the rate of 0.2 ml. per 1000 gm. of soil, while the other four pots were left as checks.

Ten days after fumigation, one tobacco seedling, which had been growing in sterile soil, was weighed and planted in each pot. After a growing period of four weeks, each plant was removed and the roots carefully separated from the soil by gentle washing. Disease assessment was based upon the net increase in fresh weight. Owing to the limited number of plants which can be used in this experimental setup, sufficient replications could not be obtained for statistical analyses. The nematodes were extracted by the Baermann funnel as before and the populations of *Pratylenchus minyus* calculated on the root weight basis. The results appear in Fig. 2.

In the steam-sterilized soil, plant growth reached a maximum at 80° F., and decreased at 90° and 100° F. In the fumigated series, the maximum

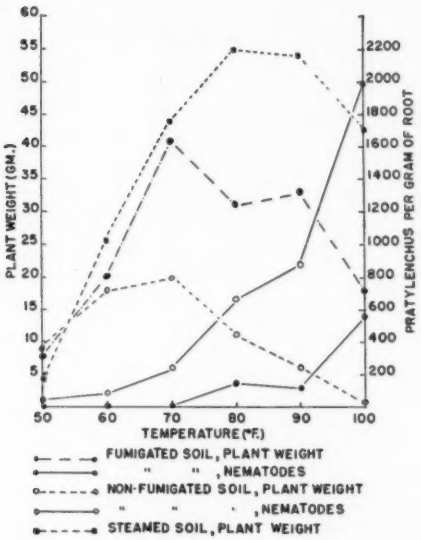


FIG. 2. The effect of soil temperature upon plant growth, as assessed by the increase in green weight and the populations of *Pratylenchus minyus*. Three soil treatments are illustrated: nonfumigated soil, fumigated soil, and steamed soil.

growth occurred at 70° F. and at higher temperatures the plants were stunted. An examination of the roots from this fumigated soil showed a marked increase in the population of root lesion nematodes correlated with the decline of plant growth at the higher soil temperatures. With ethylene dibromide a residual nematode population often remains alive after fumigation; and at the higher soil temperatures there evidently was such a rapid increase in the population that the plant growth was eventually retarded. Thus, at 100° F., the amount of brown root rot and the nematode population in the fumigated soil were roughly equivalent to those in the nonfumigated soil at 80° F.

The plants in the nonfumigated soil also reached a growth maximum at 70° F. and declined rapidly at the higher temperatures. Correlated with this decline was an extremely rapid nematode build-up. It would appear that the critical temperature below which the disease does not develop is in the 60° to 70° F. range and the maximum temperature for brown root rot and the nematode activity must be above 100° F.

Studies on Resistance to Brown Root Rot

In Ontario, selection for resistance to brown root rot of tobacco has been successful and one of the most resistant commercial varieties thus obtained is Green Briar. In brown root rot field tests conducted at Harrow, the average yield was 18 lb. per plot in the case of Green Briar as compared with 2 lb. per

plot in the case of Harrow Velvet, the susceptible variety (23). However, no significant differences in the nematode population of these two varieties have ever been reported (34).

There are indications that the resistance of Green Briar is not so marked in the more southern tobacco regions of the United States. Valleau (39) observed that the contrasts between the same resistant and susceptible varieties at Lexington, Kentucky, were not as great as at Harrow. This has been one reason for the suspicion that the nematode root rot of the southern states is not the same as the northern brown root rot. In view of the decisive effect of higher soil temperatures in increasing the nematode population, it is possible that the higher soil temperatures of the southern states might result in the nematode population increasing to the point where the tolerance of the Green Briar variety might be ineffective, and its resistance to the disease disappear.

It was decided to compare the resistance of Green Briar at two soil temperatures, viz. 70° F., which is just above the critical temperature for brown root rot in the susceptible variety, and 100° F., which represents extremely high nematode activity, although it is above the maximum temperature at which normal development of the host occurs. Corn soil infested with *Pratylenchus minyus* was used as in previous experiments. The temperature tank setup was the same as that used in the soil temperature studies. After five weeks, the increase in green weight of each plant was determined, together with the total nematodes per gram of root and percentage of *Pratylenchus minyus* which occurred. The results are presented in Table III.

TABLE III

EFFECT OF SOIL TEMPERATURE UPON RESISTANCE AND THE NEMATODE POPULATIONS

Soil temperature	Tobacco variety	Average net increase in green weight (gm.)	Average nematode population per gram of root	Percentage occurrence of *Pratylenchus minyus*
70° F.	Green Briar (Resistant)	15.4	1190	95
	Harrow Velvet (Susceptible)	6.2	1402	62
100° F.	Green Briar (Resistant)	4.9	7947	72
	Harrow Velvet (Susceptible)	0.7	4223	43

From Table III, it is observed that at 100° F., the resistant variety had made very poor growth and the plants displayed all the symptoms of severe brown root rot (Figs. 5, 6). Therefore, a breakdown of its resistance really had occurred at the higher soil temperature.

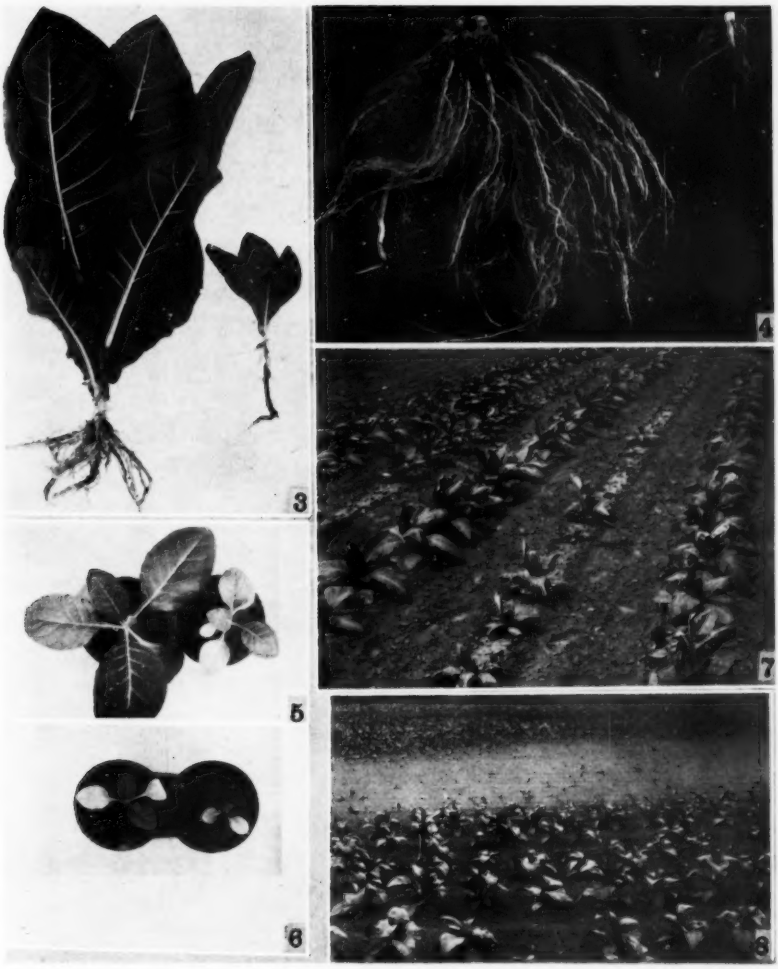


FIG. 3. A comparison of tobacco plants grown in fumigated and nonfumigated brown root rot soil. It will be observed that extreme stunting is the only aboveground symptom of the disease. Notice in the root system of the diseased plants, the tendency for new roots to be produced above the diseased region, closer to the soil surface.

FIG. 4. A comparison of tobacco roots grown in brown root rot soil; the roots on the left were grown in soil fumigated with ethylene dibromide, while those on the right were in untreated soil. Note the shallow, inadequate system and lack of lateral roots resulting from nematode attack.

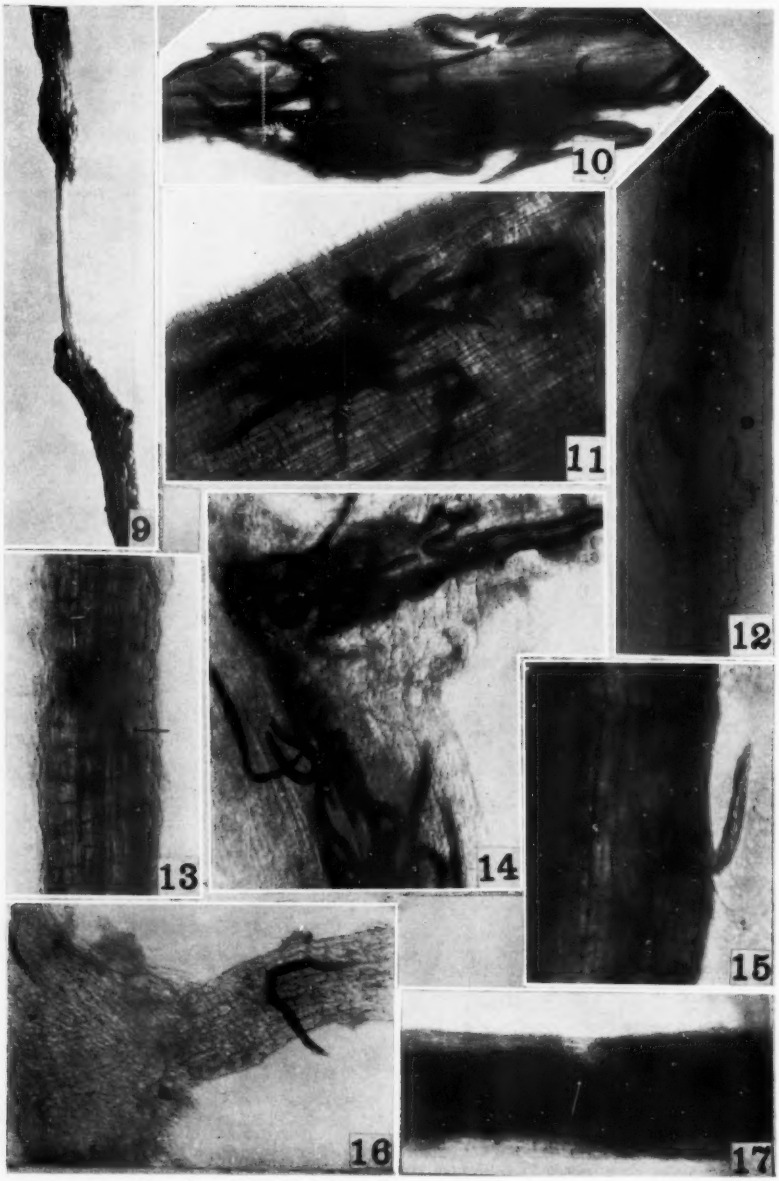
FIG. 5. Resistant variety studies. The plant at the left is Green Briar, a resistant variety; the plant at the right is Harrow Velvet, a susceptible variety. Both plants were growing in identical brown root rot soil at 70° F.

Fig. 6. Exactly as in Fig. 5, except that the plants were grown at 100° F. The disease is much more severe in the susceptible variety at this higher temperature, at which temperature also the resistance has broken down in the Green Briar.

FIG. 7. Brown root rot experimental plots. The three rows in the foreground represent three different treatments, which were, from left to right, the resistant variety, Green Briar in nonfumigated soil, the susceptible variety, Harrow Velvet in nonfumigated soil and in fumigated soil.

FIG. 8. A field of tobacco showing brown root rot occurring in a large patch, the rest of the field producing normal tobacco.

PLATE II



It has been shown previously that there is no significant difference in the green weight of these two varieties when grown in noninfested soil (23). However, an examination of the data at 70° F. shows that the susceptible variety had made less than 40% as much growth as the resistant variety, but there was nevertheless very little difference in the nematode populations of the two varieties. This lack of correlation confirmed previous experimental data (23, 34).

With increase in soil temperature, the average decline in weight of the two varieties was approximately parallel, but instead of the nematode populations being similar, as occurred at 70° F., there were 47% more nematodes in the roots of the resistant variety at 100° F., than there were in the roots of the susceptible variety. In other words, at the higher soil temperature, the roots of the resistant variety were able to support a much larger nematode population than those of the susceptible variety.

It appears, therefore, that resistance at the lower soil temperature represents a greater tolerance on the part of the resistant variety to a population level that seriously affects the susceptible variety. As the roots of both varieties appear to be attacked to the same extent at the lower temperatures, it might seem logical to assume that equal damage is done to both root systems and that resistance is correlated with the ability to produce new roots (39). However, two points tend to challenge this assumption. There is no evidence to indicate that under normal conditions the resistant variety produces a significantly more abundant root system than the susceptible one and if there is a greater rate of root production, it certainly is not great enough to account for the complete mechanism of resistance. Root destruction involves the production of necrotic tissue, and an examination of the roots of the resistant variety at 70° F. showed very little discoloration or other evidence of tissue breakdown. In the same way, the saprophytic nematode population is an indication of the relative amount of necrotic tissue available for their feeding, or as a substrate for build-up of bacteria and fungi upon which many of

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FIG. 9. An advanced lesion in a tobacco root. All of the tissues except the stele have sloughed away. The nematode alone produces a much less extensive lesion; other organisms are responsible for producing this advanced stage.

FIG. 10. A mass invasion of root lesion nematodes into a corn root which was placed beside an infested root culture for a period of 48 hr. All of the nematodes are actually within the root tissue.

FIG. 11. A "knot" of nematodes within a corn root culture resulting from a mass invasion through a single break in the epidermis. This infestation occurred in less than 24 hr.

FIG. 12. A lateral corn root showing hypertrophy resulting from heavy nematode attack.

FIG. 13. The root lesion nematodes have just completed penetration of a tobacco root culture. The arrow points to the area of penetration which represents an opening caused by the collapse of a single epidermal cell.

FIG. 14. A corn culture showing the tendency for the nematodes to move out into the lateral roots as they develop.

FIG. 15, 16. Root penetration studies. Root-lesion nematodes penetrating corn root cultures. FIG. 15 is a photograph of a living nonstained nematode.

FIG. 17. The first stage of lesion development in a cultured corn root. The lighter area marked by the arrow represents a region of disrupted cells caused by the penetration of perhaps a dozen root-lesion nematodes now feeding within the root.

them are believed to feed, and is therefore an indication of the level of root decay. However, at 70° F., only 5% of the total nematode population of the roots of the resistant variety was composed of saprophytic nematodes. But the nematode population within the roots of the susceptible variety at both temperatures and within the roots of the resistant variety at the higher temperature, when resistance was broken down, was composed of a very high percentage of saprophytic forms.

Therefore, it must be concluded that although the resistant variety is as readily penetrated and colonized by the parasitic nematodes as the susceptible variety, there is, except at high temperatures, no subsequent tissue breakdown and hence little interference with normal root function. It is postulated that resistance is not due primarily to an increased production of new roots to offset root breakdown, but is a reflection of the ability to suppress this breakdown, even under conditions of high parasitic activity.

More basic studies of the relationship between resistance and pathogenicity are underway and it appears that there might be a common relationship between varying susceptibility of the host varieties on one hand and the varying pathogenicity of the different species of the pathogen on the other.

Discussion

Although for several years the root lesion nematode has been known to be of primary importance in the brown root rot disease of tobacco, it is believed that this study is the first to establish that *Pratylenchus* is capable of initiating the typical root lesion in the absence of other microorganisms, and is thereby shown to be the primary etiological agent. This organism was found to be widespread throughout the entire tobacco producing area of Ontario and is probably indigenous. It is interesting to recall that corn, the plant most responsible for increasing the nematode populations and tobacco, the host most seriously affected, have probably been grown in some of these areas longer than any other cultivated crop.

The study of the nematode complex associated with tobacco shows that it is relatively simple and relatively constant. The changes induced in this complex are most closely associated with the previous crop history of the soil. Although representatives of nine parasitic genera and of four suspected of being parasitic occur in the roots of tobacco, only *Pratylenchus* occurs in high concentrations. It would thus seem that there exists in our tobacco soils a potentially serious parasitic fauna, which is perhaps held in check by temperature relations.

The differences in the pathogenicity of the corn and red clover nematodes, *Pratylenchus minyus* and *Pratylenchus* sp., respectively, deserve additional study. This is indicated by the fact that penetration and colonization appear to be identical in the two species, whereas the resulting parasitic populations are greater and the pathogenicity distinctly less in the case of the red clover nematode. This suggests a tendency toward the development of a host–parasite equilibrium in the case of this clover nematode.

It has been found in all cases studied that the effects of soil temperature, crop rotations, and soil fumigation in modifying the incidence of brown root rot are reflections essentially of shifts in the parasitic nematode population. There seems little reason to postulate, as has been done at one time or another, that these modifications might also be induced by changes in soil microflora, nutritive substances, or toxic substances.

It is postulated here that the tobacco plant is not the normal or even a preferred host for the species of *Pratylenchus* responsible for the disease in Ontario. Several reasons support this view. (*a*) A much better host–parasite equilibrium is established in the corn host than in tobacco. As a result of this, corn will maintain a very high population of these nematodes while showing few adverse effects. On the other hand, a relatively small population within the roots of tobacco results in serious tissue breakdown. (*b*) Root culture studies indicated that when both hosts are present, more nematodes will attack the corn and a greater population will build up within the corn than the tobacco. (*c*) If tobacco is planted in a highly-infested corn field, the meadow nematodes will penetrate these roots and build up tremendously the first year. If, however, the tobacco is grown in this same field for several years, the population of *Pratylenchus* will decline almost to the vanishing point within about three years.

The investigation into the role of temperature in the epidemiology of this disease shows that Ontario is fortunate in that the mean summer temperatures of the principal tobacco-producing areas correspond fairly closely with the minimum temperature for brown root rot. An increase of this summer temperature of only a few degrees might result in enhanced disease incidence, even in the case of a resistant variety.

The mechanism of resistance in the case of Green Briar is interesting. The fact that a root system can maintain such a very high parasitic population with little tendency toward necrotic breakdown suggests a well-developed host–parasite equilibrium which tends to minimize possible effects of the associated microflora. This equilibrium appears very similar to that maintained in corn. A very interesting question yet to be answered is whether the continuous growth of this resistant variety might not, as with the corn host, result in the maintenance of a high parasitic level in the soil concerned.

It has previously been postulated that the southern brown root rot disease differs from that encountered in the more northern regions. This assumption was based largely upon two observations: (1) that our resistant varieties are not nearly so resistant in the south, and (2) that disease severity, under continuous tobacco culture, does not decrease in the south as it does in the north. The difference in the disease incidence between the two locales is probably due to two factors: differences in soil temperatures and the fact that different species of *Pratylenchus* are involved. In Ontario, brown root rot is caused by *Pratylenchus minyus*, *Pratylenchus* sp., and probably to a certain extent, *Pratylenchus penetrans*. In the southern states, the two most common species appear to be *Pratylenchus zeae* and *Pratylenchus brachyurus*.

In this study, no special emphasis has been placed upon disease control. Control measures had been devised before the etiology was understood. In this region, adequate control can be maintained by the use of resistant varieties and the avoidance of certain crops in the rotation. Control by means of soil fumigation would not appear to be necessary at the present time.

Acknowledgments

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TEMPERATURE AND EVAPORATION AT AND NEAR GROUND LEVEL ON CERTAIN FOREST SITES[1]

By Olli Vaartaja[2]

Abstract

Microclimate, especially temperature extremes, was studied on various plots of pine and spruce sites in Finland. Thermoelement measurements were made on clear, calm summer days. Comparisons showed that the climate of the exposed sites varied greatly from that of the sites with protective ground vegetation or canopy. Very large temperature differences between the sites existed at ground-level and moderately large differences at the 5 and 20 cm. levels. At exposed sites a temperature range of 57° C. in 24 hr. was recorded in sandy surface soil, and still larger ranges seemed probable. Extreme diurnal fluctuation in temperature is evidently not closely correlated with general climate; it is favored by dryness of the soil and presence of ground cover, such as humus and fine litter, with low thermal conductivity. Maximum temperatures of 50° to 70° C. are considered of normal occurrence in thin surface soil at exposed sites in large areas of northern coniferous forests. At the same time, maximum temperatures may remain from 9° to 13° C. under the protection of canopy and surface cover, and night minima may be below 0° C. in thin surface soil. A negative correlation was found between evaporation rate and drought injuries of plants on dry sites. Root competition was a more important factor to plants than evaporation conditions, at least as shown by the measurements from large vessels.

Introduction

Besides light, the ecological factors of the greatest importance to plants are temperature and moisture. Although even descriptive studies of these elements of microclimate would be useful, little exact work has been done in this field. The reasons for this situation lie in the difficulties presented by the technical problems of measurement and, especially, by the vast variety of microclimates. These microclimates vary with geography, general climate, topography, differences in the plant communities themselves (species, age, and structure), and locality within the communities, e.g. with the strata.

The methods of ordinary climatology have drawbacks in microclimatology. It is true that the first helpful investigations in this field were made with the usual instruments at the standard observation height of approximately 1.8 meters and, as a rule, with three standard observations daily over a period of several years. For instance, one learns the following from Müttrich's (10) records taken over the best part of 15 years: the air temperature at a height of 1.8 meters is lower in a forest stand than in an open place by day, particularly in the summer; night and winter produce a reverse situation; near Eberswalde in Germany, in certain beech stands, the daily temperature amplitude in June was 4.4° C. less than that in the open; this modifying influence of the forest on temperature variation was not so marked in spruce (3.8° C.) and pine (3.0° C.) stands.

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Contribution No. 156, Forest Biology Division, Science Service, Canada Department of Agriculture, Ottawa, Canada.

[2] *Forest Biologist, Forest Pathology Laboratory, 309 Birks Building, Saskatoon, Saskatchewan.*

Other investigations have shown that the modifying effect of the stand on temperature is greater at or near the soil surface, and that evaporation is much reduced in a forest stand. For instance, in a dense virgin stand in Idaho the temperature in a humus surface from June to August, 1931 to 1933, was 49.9° F. (27° C.) ± 1.5° F. less than that in an open place (7). The corresponding difference in evaporation from "atmometers" was (163.0 − 40.0 =) 123 ± 11.2 gm.

In modern microclimatic investigations there is a trend towards the use of special methods, detailed study of the climates of very small spaces at a few typical localities, and limitation of observations to only a few days of typical weather conditions. Geiger (5) has reviewed several such studies. Franssila, for instance, studied only one plot for a few days, but was able to reveal the whole temperature economy of the plot. This trend is justified, in view of the variation in the conditions, for the understanding of climate as an environment for plants and with regard to the physical factors composing it.

With the purpose of exploring some aspects of the climate of northern coniferous forests, the present study was planned along the following lines: only the environment at or near the soil surface was investigated; it was found necessary to use the thermocouple and to investigate its application (13); although no comparable instruments existed for humidity measurements, efforts were made to study evaporation; temperature was studied on a few typical days, special attention being paid to the extremes; evaporation was studied only during a drought period on dry sites; the sites studied were also limited to a few extremes, to dry pine (*Pinus sylvestris* L.) heaths and to a few types of moderately moist spruce (*Picea abies* Karst.) sites in Finland.

The study was confined to extremes in various ways on account of the large variety from which samples had to be chosen, and because of the importance of extremes as minimum factors in plant ecology. The data will be presented mostly as representative examples and comparisons. Statistical treatment was applicable only in a few instances.

Methods

The Sites

Tables I and II give the localities and characteristics of the sites where vertical temperature distribution was investigated. These sites were all situated in South Finland. Some temperature measurements were made at other localities in South Finland and at Rovaniemi in North Finland near the Arctic Circle. Evaporation was studied only at a heath in South Finland.

The climates of various dry sites (plots 1 to 8) that were much exposed were studied mainly on a wide homogeneous flat heath. Disturbances due to the moderating influences of lakes or other waters were thus avoided.

Where ground vegetation existed, it was, as a rule, sparse and low. On plot 7 dense *Calluna vulgaris* hummocks (height 28 ± 0.1 cm.) were common. Plot 13 was covered by dense *Calamagrostis arundinacea* with a uniform height of 1.2 meters. The most important species on dry sites were: *Cladonia silvatica*,

TABLE I

CHARACTERISTICS OF THE MAIN INVESTIGATION SITES

Plot	Dominant trees		Surface cover[1]	Description
	Species	Height (m.)		
A. Sites at Ruovesi aerodrome, 61° 40′ N.:				
1	(Pine)[2]	0	Humus	Burnt 4 years previously
2	Pine	15 to 20	Vegetation	Mature stand, crown project area 56%
3	Pine	(2 to 3)[3]	Sand	Gap with no seedlings, 3 m. wide in dense regeneration
4	Pine	15 (2 to 3)[3]	Sand	Gap with no seedlings, 5 m. wide with over-topping trees
5	—	—	Gravel	Aerodrome
6	Pine	0.5 to 2	Vegetation	Sparse regeneration
7[4]	(Pine)[2]	0	Vegetation	Hummocky *Calluna* stand (old burn)
8	Pine	2.5	Vegetation	Overdense regeneration
B. Sites at Hyvinkaa aerodrome, 60° 40′ N.:				
9	Spruce	12	Moss	Dense stand, crown project area 90%
10	(Spruce)[2]	(12)[3]	Humus	Freshly cut opening 15 m. from uncut stand
11	(Pine)[2]	0	Vegetation	*Calluna* stand, half mile from 9 and 10
C. Sites at Viik Experimental Farm 60° 20′ N.:				
12	Spruce	25	Litter	Mature stand, crown project area 95%
13	(Spruce)	(25)	Grass	Freshly cut opening, 25 m. from uncut stand

[1] *Around the bared spot where temperature measurements were made.*
[2] *Before the fire or cutting.*
[3] *Around the gap or opening.*
[4] *Standard plots for comparative measurements.*

TABLE II

PERCENTAGE OF VEGETATION AND OTHER COVER ON PLOTS[1], FOR THREE STRATA

Plot No.:	1	2	4	6	7	8	9	10	12
Lichen	0	73	Tr.[2]	74	60	70	Tr.	Tr.	0
Moss	Tr.	27	Tr.	12	20	6	90	Tr.	20
Humus	70	0	10	7	15	0	0	80	0
Litter	5	Tr.	50	3	Tr.	24	10	20	80
Sand	25	0	40	4	5	0	0	Tr.	0
Ericaceae	22	27	10	39	45	61	20	10	Tr.
Conifer regener.	Tr.	3	Tr.	12	Tr.	0	0	Tr.	Tr.
Deciduous regener.	Tr.	0	0	0	Tr.	0	0	Tr.	Tr.
Herbs	Tr.	0	0	0	0	0	Tr.	Tr.	Tr.
Grass	0	0	0	0	0	0	Tr.	Tr.	Tr.
Layer 2 together	22	30	10	51	45	61	20	10	Tr.
Layer 3 (canopy)	0	56	(100)	0	0	100	90	0	95

[1] *Plot 5—gravel only; plot 13—grass only; plot 3—litter 20% and sand 80%. Plot 11 was similar to plot 7.*
[2] *Trace.*

C. rangiferina, Polytrichum piliferum, Calliergonella schreberi, Dicranum undulatum, Dicranum spp., *Vaccinium vitis-idaea, Calluna vulgaris;* on good sites: *Hylocomium proliferum* and *Vaccinium myrtillus.* The spruce stand around plot 12 had only a very low and sparse vegetation, principally of *Maianthemum bifolium.*

The study of the climate of low ground vegetation is a complex problem because of the differences in the various minute interstices, e.g., between the sunny and shaded sides of leaves and stems. Only a few temperature measurements were made in such vegetation. Instead, seed spots of 30 to 40 × 40 cm. were studied. These spots were bared to the mineral soil in each plot. On plot 13 the high grass was removed from a circle 1.5 meters in radius.

In every plot the soil consisted mostly of sand.

Temperature Measurements

Thermoelements with junctions 0.2 to 0.4 mm. thick were used. A detailed description of the method has already been published (13).

When the temperature of any exposed soil surface was measured, the junction on the soil may be much warmer than the wires immediately above in the air. This state will cause heat conduction through the wires and errors in the records. Special attention was given to the analysis and correction of this conduction error. The correction was made cautiously with the result that the maximum temperatures given later tend to be too low rather than too high.

The error was reduced by making the parts of the wires connecting the junction of very thin material (0.02 to 0.15 mm. thick). In some types of thermoelement the thin parts of the wires were insulated by two thin glass tubes put one inside the other. The tubes reduced the error by only a small amount, but strengthened considerably the otherwise too delicate thermoelement.

By the use of these thermoelements, temperatures were observed in the surface soil at several spots as well as at heights of 1, 5, 20, and 100 to 180 cm. in the air, all during periods of three to five minutes. However, when considerable unrest in temperature (irregular fluctuation (5, p. 42)) occurred, an observation usually lasted one-half to one minute and the average value was estimated. To lessen the effect of unrest the junction was made to vibrate in the air. Soil temperature values are based on several observations, and air temperature values on at least one or two observations.

At a height of 180 cm., Assmann aspiration thermometers were also used. Minimum temperatures were recorded by Fuess alcohol minimum thermometers.

Other Measurements

Evaporation was studied by measuring the reduction in the amount of water kept in shallow zinc containers. The height of the containers was 5 cm. and the surface 500 sq. cm. On most of the plots the evaporation containers

were placed at two or three different heights. The protective effect of slash or ground vegetation was also studied. The precipitation was observed at each plot and a corresponding correction made to the evaporation values. Rain gauges were inserted in the ground to avoid errors due to turbulent air movement. However, only the rate of evaporation recorded during periods of low rainfall (less than 1 cm./10 days) was considered reliable.

Relative humidity of the air and wind movement were studied at some plots on dry sites. Wind speed was measured by cup anemometers at heights of 1.8, 0.4, and 0.2 meters. Wind data were calculated from repeated records obtained over periods of 10 min. The comparisons of wind conditions on various plots were based on simultaneous records at two places. Humidity was measured by Assmann aspiration psychrometers.

Effects of Clouds, Rainfall, and Wind

When radiation has been minimized by cloudiness, or air has been mixed by wind, or the thermal properties of the soil have been equalized by rain water, extreme temperatures will not develop and various forest stands may show similar temperatures. For this reason, observations were made mainly during clear, calm weather at least three days after the occurrence of rain. This was especially desirable when surveying the small differences between plots 1 to 8, most of which were rather exposed. The weather during the study of these plots is shown in Table III.

TABLE III

CERTAIN WEATHER CHARACTERISTICS DURING THE STUDY PERIOD ON THE STANDARD PLOT (NO. 7)

Precipitation		Weather at 3.00 p.m.				
Date 1946	Amount in mm.	Dates of comparative measurements 1946	Temperature at 1.8 m., ° C.	Relative humidity at 1.8 m., %	Wind velocity at 1.8 m., m./sec.	Cloud index[1] (0–10)
June 1– 6	51.5	June 9	19.2	22	2.0	2
10–16	9.8	24	25.4	29	3.1	1
18–20	13.2	July 5	26.3	24	2.5	2
27–28	9.9	13	23.1	17	1.4	0
July 7–8	8.7	18	25.7	19	4.0	3
10	0.6					

[1] *Tenths of sky obscured by clouds; exclusive of thin cirrus.*

Cirrus clouds did not have much influence on the microclimates. Despite cirrus clouds, surface temperatures as high as the following can occur:

	Maximum, ° C.	Average at noon, ° C.
(*a*) 1 to 3 cm. thick humus and litter	64	62
(*b*) 2 mm. thick forest humus	59	52
(*c*) White sand (slightly moist)	42	38

These temperatures were recorded on exposed places in thin surface soil on July 19, 1949, on a seed-tree area of *Vaccinium* type at Kettukangas at Vilppula near Ruovesi. The air temperature at 1.8 meters was 20° C.

At the same time in a swamp at a distance of 200 meters, the effect of soil moisture was revealed by the following maxima in surface soils:

(*a*) Dry *Carex–Sphagnum* peat piled out from a ditch 61° C.
(*b*) Moist patch of the same peat at a slightly lower position, 1 meter distant 31° C.

Evidently the water caused considerable thermal inertia in soil (*b*) as compared with the similar dry soil (*a*). Water increases the thermal capacity and conductivity of soil and cools it by evaporation. The situation described above is an extreme case of the inertia effect of moisture on soil temperatures. The inertia effect of water was mostly small, if it existed, when the climates of ordinary plots were studied, owing to the fact that the main period of investigation, June 9 to July 24, 1946, was practically rainless (Table III). The precipitation of June, 1946, was 84.4 mm. on the standard plot. This is 7 mm. below the normal value (8). In July, rainfall was only 28.9 mm., this amount being 48 mm. below normal.

In the peat at plot (*a*) above, the capillary movement of water from moist deep layers had been artificially interrupted. A surface of natural undisturbed forest humus may also be dry and show high temperatures within a few days after rain, the deeper soil remaining moist. This was the case in the following temperature records on a seed-tree area of *Vaccinium* type (at Hyryla, north of Helsinki, July 1, 1949, at noon):

(*a*) 2-cm.-thick humus with dry surface 63° C.
(*b*) Wet sand under the humus 20° C.
(*c*) Nearby sand with dryish surface 51° C.
(*d*) Air at 1.8 m. 26° C.

Cumulus clouds changed surface soil temperatures in the following manner:

Time	Position of cloud	Temperature in burnt humus, ° C.
13.45	Far from sun	67
13.47	Near sun	69
13.48	Beginning to cover sun	Changing
13.50	Covering sun	38
13.52	Covering sun	36
14.05	Beginning to show sun again	Changing
14.09	Far from sun	61
14.15	Far from sun	63

The change in five minutes (14.47 to 14.52) was 33° C. The temperature rise, after the cumulus clouds had passed, did not quite reach the previous peak because radiation was decreasing at that time of day.

The above measurements were made on a seed-tree area of *Vaccinium* type on a 10° slope with a southwest exposure at Tuusula near Helsinki, July 2, 1949.

Many other records showed a similar sudden temporary effect of cumulus clouds. A slight rise in temperature was often found just as cumulus clouds approached the sun. The explanation probably lies in the increase in radiation by reflection from the side of the cloud.

Actually, scattered small cumulus clouds do not change microclimatic conditions much from those which would prevail on a completely clear day. However, in comparisons of different sites made later in the study, observations affected even temporarily by thick clouds were discarded.

The temperatures of most sites at or near the ground were only slightly influenced by wind. Individual temperatures as high as 57° C. were recorded in the exposed sand (plot 7, June 18, 1946) despite a wind speed of 4.0 meters/sec. at a height of 1.8 meters. A wind as strong as or stronger than this seemed, however, to increase the dispersion of individual records of surface soil temperatures and to lower the average temperatures somewhat.

Wind on Various Sites

Forest stands are obviously impervious to all but the strongest winds. This was the case with the pine and spruce stands studied (plots 2, 9, and 12).

The retarding effect of sparse low sapling stands on wind is less obvious. This effect was studied by comparing the wind speeds on plot 7 (*Calluna*) and on the neighboring plot 6. The saplings around this latter plot retarded winds of moderate speeds to 89% at the height level of 1.8 meters. At lower levels and close to each sapling, the retardation was much greater.

The retardation of wind near the ground was studied by comparing the wind speed at three levels on most of the sites. The wind speeds at the two lower levels are indicated below as percentages of the speeds at 1.8 meters.

Plot	Stand of	0.4 m., %	0.2 m., %
1	(Burnt area)	67	55
2	Mature pine	41	37
3	Gap among saplings	60	24
4	Under pine group	63	50
6	Sparse saplings and *Calluna*	42	15
7	*Calluna*	56	34

These measurements were made above an open seed spot during moderate or strong winds; within the ground vegetation, where such vegetation existed, the reduction was probably much greater. The anemometers often stopped when an effort was made to measure wind speed at the height of 0.2 meters, even during moderate winds. On all the plots except that at the aerodrome and that at the burnt area, plots 5 and 1, wind at such a low level seldom exceeded 1 meter/sec. Evidently the reduction in wind speed was great close to the ground, especially where the ground was covered by vegetation.

Similarly, Stocker (12), in Germany, has found that the vegetation retards wind greatly. In a *Calluna* stand more vigorous than that on plot 7 of the present study, he found that the wind velocity at a height of 0.4 meters was 63% of that at 1.8 meters.

Temperatures Under Soil Cover

Soil temperatures were studied by a small, sensitive mercury thermometer inserted into undisturbed soil horizontally. Table IV shows the results obtained.

TABLE IV

SOIL TEMPERATURES OF VARIOUS SITES AT A DEPTH OF 5 CM.,[1] JUNE 1, 1946

Plot	Site		Mean and standard error of 15 measurements, ° C.
	Vegetation	Soil cover	
5	None (aerodrome)	Gravel	12.6 ± 0.04
3	Gap among saplings	Loose sand	12.0 ± 0.14
4	Under group of pines	Loose sand	11.1 ± 0.08
6	Saplings, sparse	Lichen, ½ cm. humus	10.9 ± 0.11
7	*Calluna* hummocks	Lichen, ½ cm. humus	10.8 ± 0.29
2	Mature pines	Litter, moss, 1 cm. humus	8.2 ± 0.11
8	Saplings, dense	Litter, lichen, 1 cm. humus	8.0 ± 0.06
—	Poles, dense[2]	Litter, thick moss, and humus	7.4 ± 0.11

[1] *From the mineral soil surface.*
[2] *Of pines, near plots 6 and 7.*

The temperatures of the plots with different soil covers differed significantly from each other, though only by a few degrees. Evidently litter, moss, and humus had retarded the warming of soils.

The difference between plots 5 and 3 is probably mostly due to the better thermal conductivity of the gravelly soil on the aerodrome as compared with the poorer conductivity of the loose dry soil on plot 3.

These temperatures were measured on a foggy overcast day when the general daily rhythm of temperature hardly penetrated the soil. However, approximately the same relationships between the soils prevailed on clear days. At a depth of 15 cm. the same order was also found in soil temperatures. On June 1, 1946, these were roughly 1° C. cooler than those at the depth of 5 cm.

The effect of soil cover on the soil temperature was also studied experimentally. Flat, homogeneous, sandy soil near Hyvinkaa aerodrome was kept covered with various natural covers. Each of the four covers had a surface of 4 sq. meters and they were separated by 2 meters. Thermocouples with junctions 15 cm. long were inserted horizontally at the middle of the covers at various depths.

The records obtained at noon, June 26, 1951, illustrate the protecting effect of the various covers:

	Litter layer, ° C.	Sand surface, ° C.	Depth of 10 cm., ° C.
(*a*) Thick pile of slash with the undisturbed cover of a typical open *Calluna* heath (near plot 11)	15.6	13.9	10.4
(*b*) Moss and humus cover from a typical spruce stand of *Myrtillus* type (from plot 9)	26.3	17.8	13.7
(*c*) As (*a*) but without slash	28.9	18.3	15.6
(*d*) Denuded area (sand)	—	35.8	16.4

The moss mat (*b*) was nearly 10 cm. thick and thriving two weeks previously when it was moved from a nearby dense stand, but it gradually shrivelled under the exposure. It probably lost part of its protecting effect. The dominant species of the mat was *Hylocomium proliferum*.

Under both a fresh ground cover and a canopy on sand layer, low temperatures were recorded. Though not quite comparable, the following records illustrate the tendency found:

	° C. at noon
July 11, 1951, at Hyvinkaa:	
(*a*) Open *Calluna* heath (plot 11)	20.1
(*b*) Mature pine stand, similar to plot 2	12.8
(*c*) Dense spruce stand (plot 9)	12.5
July 23, 1951, at Helsinki:	
(*d*) Opening of very dense spruce stand (plot 13)	12.5
(*e*) Very dense stand 50 meters from (*d*), (plot 12)	12.2
July 23, 1949, at Rovaniemi:	
(*f*) Cut-over area near a spruce stand	12.6
(*g*) Sparsely stocked spruce stand of *Hylocomium–Myrtillus* type near (*f*)	9.0

By midsummer, the only considerable warming of the soil that had taken place was at such places as plot (*a*) where the lichen cover of the soil was exposed, broken, and thin. The humus of this site was only about 0.5 cm. thick. At plots (*b*) to (*e*) the thickness of the humus varied from 2 to 5 cm. and the lichen or moss mat was vigorous and continuous. At plot (*g*) the soil cover was especially dense and thick, altogether nearly 20 cm.

The data presented here, as in similar comparisons not published, suggest that warmth penetrating the forest soil during summer varies greatly according to the quality of the surface cover and canopy. These data also suggest that soil temperature may be low, even in the middle of the summer, and is thus to be considered as a factor of great silvical importance.

Homen, Mork, Ronge, and Ångström (reviewed by Aaltonen (1)) have studied soil temperatures under conditions resembling those above. Their results showed the soil to be cool under the combined influence of canopy and ground cover. The intensive investigation of Herr (6) in Germany also emphasized the effect of the cover on forest soil temperatures.

Temperature of Various Soil Covers

The surface layer of a thick forest humus in the case described on page 764 reached an average temperature of 24° C. higher than that of whitish sandy soil, both being exposed to insolation. Thin humus warmed up to 10° C. higher than the sand.

In other cases this difference between humus and sand temperatures was 11° and 8° C. (Fig. 2), 12° C. (page 765), 5°, 5°, and 11° C. (13).

The following temperature records for thin surface material were made under the conditions mentioned on page 765:

	° C.
Humus, 2 cm. thick	63
Piece of rotten gray log	54
Humus, 2 mm. thick	52
Brown sand	51
Air at a height of 1.8 meters	26

Another similar comparison (the conditions as described on page 765) yielded the following maxima:

	° C.
Humus and some litter, 2 cm. thick	70
Humus, 3 mm. thick	59
White sand	52
Air at a height of 1.8 meters	24

Besides the difference between temperatures of exposed humus and sand, differences exist in various mineral soils (14). The finer the particles, the hotter are the exposed surface soils of sand and gravel. Fig. 2 illustrates the difference (7° C.) between temperatures of loose and compact sandy soil.

The factors on which these temperature differences are based are differences in albedo (reflection ability), thermal capacity, and thermal conductivity of the soil.

Conductivity differences were evidently sufficient to account for the fact that a negative correlation was usually found between the maximum temperatures of a thin surface layer and the temperatures deeper in the soil. Many sites with high maxima on the surface, soils with humus for instance, were cool at deeper levels; those with low maxima on the surface, such as the bare aerodrome (Table IV), were warm at deeper levels, given the same exposure.

The temperatures of seemingly uniform surface soil were found to vary greatly from one point to another (13). This variation was also studied on a

clear-cut area of *Hylocomium–Myrtillus* type at Rovaniemi, near the Arctic Circle, on the afternoon of July 23, 1949. The variation in 20 records resulted in a standard error of ± 1.8° C., the mean in the surface humus being 59.9° C. The absolute maximum was 68° C. The high dispersion was caused partly by differences in conductivity errors but also by small irregularities in the albedo and by thermal properties varying from spot to spot in the soil.

The surfaces of most forest sites were composed of ground vegetation so irregularly shaped that it was impossible to study their temperatures thoroughly. Temperature difference of exposed lichen and sand was measured by the use of a dark pine seed as a test object (5, p. 70). By placing a pine seed, inside of which a thermojunction had been inserted, on the exposed surfaces of *Cladina* and sand in turn, the sand surface was found to be cooler. The average difference of a measurement series was 12° C. On the other hand, when this seed was placed on a shaded lichen surface, its temperature was often 30° C. lower than on an exposed lichen surface.

Sometimes the surface of forest sites is a relatively homogeneous humus but with small cracks through it to the underlying sand. The temperature differences between such a humus surface and the sand surface in the cracks were measured at Rovaniemi, in the Arctic Circle, on July 22, 1949. The humus was 0.5 to 2 cm. thick, typically cracked and exposed on a clear-cut area of *Empetrum–Vaccinium* type. The mean temperature difference (± standard error) between the humus and the sand in the cracks was 27 ± 2.0° C. In an extreme case the humus showed 60° C. and the sand in a crack 29° C.

The nocturnal minimum tended to be lower in forest humus than in sand but the differences were not very great (Fig. 4).

The temperatures of *Cladina*-covered spots and those denuded to the sand were compared by minimum thermometers laid on these surfaces at night. The average temperature of the 10 coldest nights between July 15 and August 15, 1946, was 2.7° C. higher on sand than on lichen.

All these studies suggest the great influence of soil cover on surface temperatures. The most extreme conditions evidently exist in exposed humus or litter. In exposed sand the conditions are somewhat less extreme. The differences between these cover types are large (even 24° C.) in maxima, but small in minima. If any vegetation exists, differences in exposure cause great maximum differences from spot to spot. Even in soils without vegetation the surface soil maxima may be considerably dispersed.

Observations on Drought Injuries

Calluna plants and pine saplings showed discoloration and needle cast on various plots in midsummer, 1946. On microscopic examination, no other cause but the apparent drought could be found to account for this condition.

Table V shows the severity of the injury on various sites. The degree of injury denoted as "5" means that about half the needles or leaves were cast or discolored from pale yellow (mostly older pine needles) to rusty red (part

TABLE V

DROUGHT INJURY ON PINE AND *Calluna* AT VARIOUS SITES IN 1946

Plot	Vegetation	Injury	
		Time of start	Degree (0 to 5)
5	Few single seedlings (aerodrome)	—	0
1	Few single seedlings (burnt area)	Late	1
7	*Calluna*, few saplings	Late	1
6	*Calluna*, sparse saplings	Late	3
3	Gap densely surrounded by saplings	Early	4
8	Dense saplings	Early	5

of the needles and *Calluna* leaves). "0" means no visible symptoms but perhaps a very faint yellowish shade in the normal green color.

The denser the vegetation, the more severe were the drought injuries. Evidently dense stocking caused intense root competition and hence dryness of the soil. Thus, the injuries reflected the extent of drying in the soils.

Between the sparse saplings and in the gap in the dense sapling stand, the soil was invaded by pine roots even at a distance of several meters from the saplings. In this way the saplings influenced the soil condition, and hence the microclimate, at a considerable distance.

Comparison of Temperatures on the Plots

A critically chosen part of the thermoclimatic data was illustrated graphically by 166 curves. The most representative of these curves have been copied in Figs. 1 to 7.

Fig. 1 gives the graphic patterns of daily temperature rhythm on two plots at a height of 1 cm.

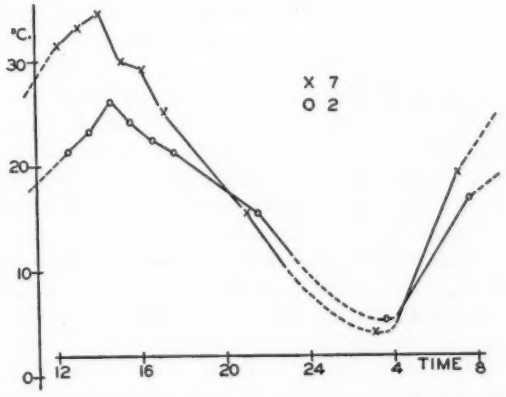

FIG. 1. Daily temperature fluctuation at a height of 1 cm. above denuded sandy spot at two plots, July 13, 1946.
7 = *Calluna* plot; 2 = pine stand.

In Figs. 2 to 7, each point on a curve represents the average temperature at a certain time at a certain height (in surface soil, and in air at the levels of 1, 5, 20, and 180, or rarely, 100 or 150 cm.). Temperature unrest (page 763) in the air made it somewhat difficult to obtain an average at any given level. Though this difficulty may have caused some irregularity in a few afternoon curves, it has been overcome in most cases. The same is true of the soil temperatures in the figures. These temperatures are averages and hence do not show the highest individual maxima mentioned in other sections.

Irregular temperature variations in the air and in the soil surface occurred only in the afternoons and could not mask the great differences between different levels at that time. Consequently, a comparison between the points of each curve is illuminating.

Most of the curves in a figure are also comparable with each other. However, the following facts must be taken into consideration. The measurements were not made simultaneously, but at intervals of 15 to 30 min. Some of the late evening curves are not comparable with each other since temperatures altered rapidly at that time. These curves (Figs. 3, 4, 5) serve only to illustrate the temperatures on each plot at various heights. In Fig. 2 the late evening curves are comparable with each other because the successive measurement series were made at sufficiently short intervals and an interpolation correction was made.

Unpublished afternoon curves showed that, in general, temperatures changed slowly at the times of maxima. Therefore, and because interpolation corrections (Figs. 2 to 5) were made, all the afternoon curves published in a figure are comparable.

At dawn, about the time of the minimum, temperatures also changed slowly. These curves are at least roughly comparable in a figure.

Comparisons between curves of different figures (days) can be made only as approximations.

Temperature at 1.0 to 1.8 Meters

At this height level, air of most sites was fairly free to move and thus convection largely equalized the temperatures. Various sites showed about the same temperatures at this level; the greatest differences between sites, in spite of calm weather, were in general about 2° C., in Figs. 2 to 6. A greater difference of 5° C. was noted in a single case between the *Calluna* plot (No. 11) and the spruce stand (plot 9).

It may be that the differences in temperature between grown up stands and the open at 1.8 meters are greater than in Figs. 2, 5, and 6, if the central parts of large stands are compared to the open. The plots were relatively near the stand margins.

The Shapes of the Curves

While the plots differed little in temperature at a height of 1 to 1.8 meters, many of them differed greatly in the temperature of the surface soil. In

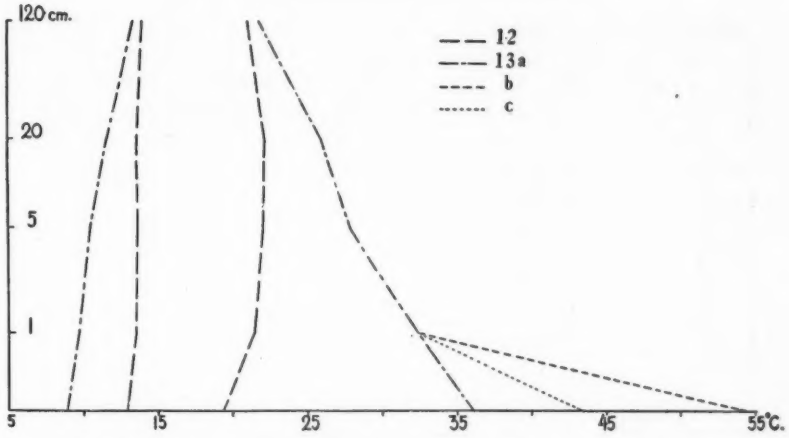

FIG. 2. Late evening (left) and afternoon temperatures at two plots, July 17, 1951. 12 = spruce stand; 13 = opening in the spruce stand; *a* = sand; *b* = loose sand; *c* = humus.

general, these differences were reflected to some extent in the air temperatures at a height of 1 cm. At the height of 5 cm. and especially of 20 cm., this influence was much less marked, when it existed at all.

The curves usually showed the characters of either "night type" (cf. Geiger (5, pages 6 and 65)) with a minimum at the ground (Figs. 2, 4, 7) or "irradiation type" with a high maximum at the ground (Figs. 2 to 7). Several exceptions were found.

Many of the dawn curves (Figs. 3, 4, 5) and a few of the early night curves (Fig. 3) had a slight minimum at a height of 1, 5, or 20 cm. This peculiarity was probably caused by the following factors: (*a*) The sandy plots where all the measurements were made cooled less rapidly than the surroundings with humus, litter, and lichen cover. (The relatively great thermal inertia of sand soil was reflected also in the temperature records made 1 cm. above sand and nearby lichen. The minimum thermometers always showed a lower minimum above *Cladina*. In July, the average difference between these two spots was 2.0° C.) (*b*) Radiation occurred from the ground vegetation and resulted in the cooling of the air at levels of 5 to 20 cm. (*c*) Low fog and condensation probably produced more heat than was lost from the ground by radiation and this, in addition, was reduced by the back radiation from the fog. At dawn, after intense cooling, a fog layer, though thin, low, and sometimes hardly visible, was common on the plots in spite of the dry character of the area.

Furthermore, the afternoon curves for the spruce stands (Figs. 2 and 6) did not show the irradiation pattern. The canopies were too dense to allow warming of the ground by radiation, but this took place by convection from warmer surroundings.

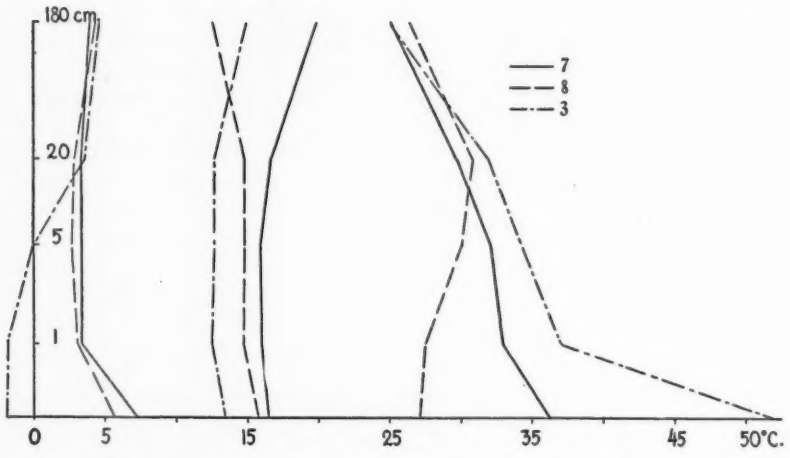

FIG. 3. Dawn (left), late evening (middle), and afternoon temperatures at three plots, June 24, 1946.
7 = *Calluna* plot; 8 = dense pine saplings; 3 = gap among pine saplings; surface soil temperatures measured on denuded sandy spots.

The curves for the dense sapling stand (plot 8, Fig. 3) resemble those for the *Antirrhinum* stand studied by Geiger (5). In both cases, the stands were not very dense but were sufficiently so to make the air layer inside them warm by day and cool by night.

Temperature at the Ground

Thermal characters of various sites were best illustrated by their temperatures in surface soils.

Calluna plots showed high afternoon and low nocturnal temperatures during clear weather (Figs. 3 to 5 and plots 7 and 11). However, the extremes were not as intense as on some other exposed plots. This fact may have been attributable mainly to two factors: (1) The vegetation was not very dense or high, so it allowed the plot to cool considerably by turbulent mixing (5). (2) The root activity of the sparse vegetation did not dry out the soil very readily as was indicated by the low incidence of drought injury (Table V). When the measurements for Figs. 3 and 7 were made, the soil of this plot was moister than that of plot 4. This high moisture caused high thermal inertia, preventing this plot from showing such high maxima as drier plots.

In midsummer, however, maxima over 50° C. were often recorded in the surface sand of this plot. At the same time low night temperatures were recorded with minimum thermometers 1 cm. above (*a*) plot 7 (sand) and (*b*) the surrounding lichen mat. The following incidence of different minima was found:

		Below 0° C.	0° to 5° C.	Above 0 ° C.
			No. of nights	
June 10 to 30, 1946	(*a*)	2	6	13
	(*b*)	2	7	12
July, 1946	(*a*)	3	10	18
	(*b*)	8	12	11
August 1 to 10, 1946	(*a*)	0	6	4
	(*b*)	3	5	2

The temperatures on the open burnt area, plot 1, and aerodrome, plot 5, differed little from those on the *Calluna* stand, plot 7. The soil temperatures were somewhat more extreme on the burnt area (Fig. 4) and less extreme on the aerodrome. The differences were probably caused by the low albedo due to the blackness, by the bareness of the surface on the burnt area, and, on the other hand, by the good thermal conductivity due to the compactness of the soil of the aerodrome (see page 769).

Other measurements (e.g. those on pages 764, 765, and 769) suggest a similar or slightly greater range in temperatures of open cut areas as compared with the *Calluna* plot (curves in Figs. 3 to 7). The prerequisites for such extreme diurnal ranges are evidently the great exposure due to scarcity of ground vegetation and the relatively good insulation capacity of the soil cover. Thus, extremes of temperature can be considered a general characteristic of open cut forest areas.

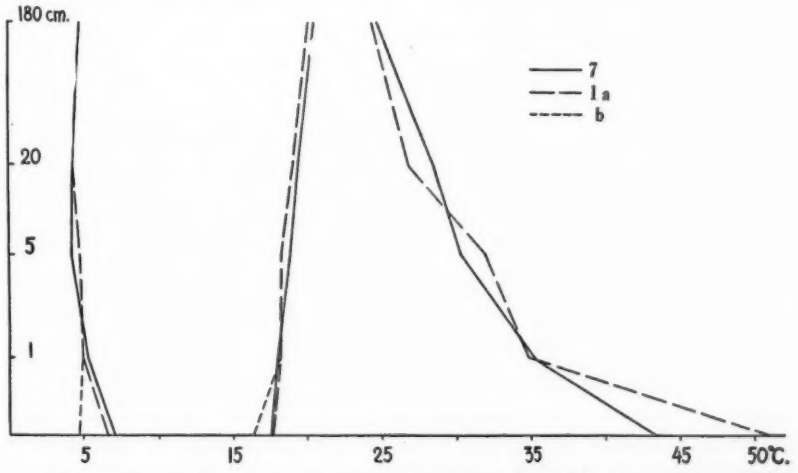

FIG. 4. Dawn (left), late evening (middle), and afternoon temperatures at two plots, July 5, 1946.
7 = *Calluna* plot; 1 = burnt area; *a* = sand; *b* = humus.

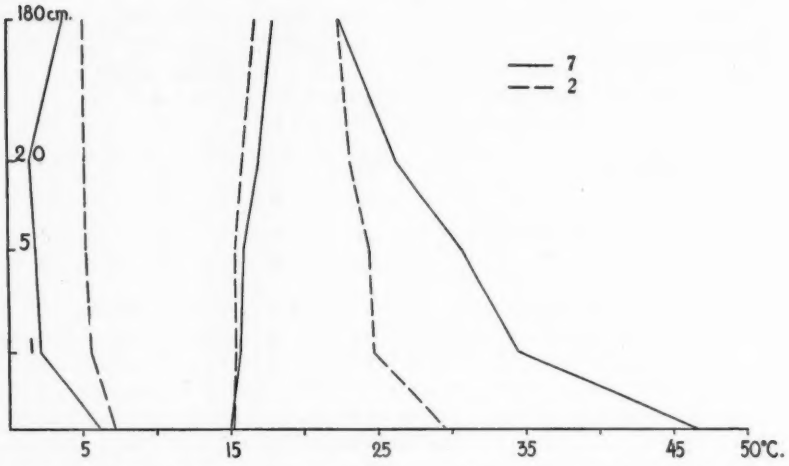

FIG. 5. Dawn (left), late evening (middle), and afternoon temperatures at two plots, July 13, 1946.
7 = *Calluna* plot; 2 = pine stand; surface soil temperatures measured on denuded sandy spots.

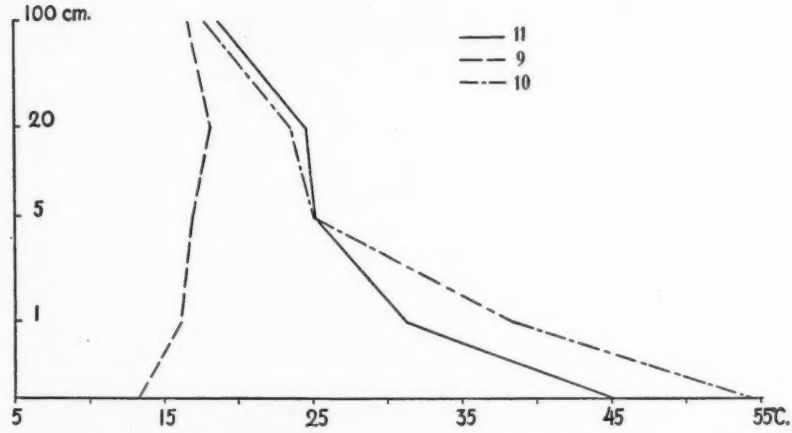

FIG. 6. Afternoon temperatures at three plots, June 13, 1951.
11 = *Calluna* plot; 9 = spruce stand; 10 = opening in the spruce stand; surface soil temperatures measured on denuded sandy spots.

The opposite situation, with a much lower range of temperature, at plots 12, 2, and 9 (Figs. 2, 5, and 6) can obviously be considered to prevail under canopies of fully stocked stands. The degree to which extremes are prevented varies greatly, depending on the closure of the canopy.

The pine stand of plot 2 represents a very poor site which cannot support a completely closed canopy. In this stand the temperature distribution near the ground is of the irradiation type. The spruce stands of plots 9 and 12, on the other hand, were fully closed and there the curve showed the peculiar shape by day that was discussed earlier.

The difference in maximum soil temperature between the mature pine stand and the *Calluna* plot was 23° C. (July 13, 1946). The corresponding difference between the spruce stand and the *Calluna* plot was 32° C. (June 13, 1951). Although not quite comparable, these differences, 23° and 32° C., illustrate roughly the dissimilarity of a pine stand with rather open canopy and a very dense spruce stand. Most northern coniferous stands fall into categories between these.

Intermediately closed stands may show irregular temperature conditions at the ground, some patches being more exposed than others to direct radiation through gaps in the canopy.

The bare plot 3 which was densely surrounded by saplings showed the most extreme temperatures (Fig. 3). On the thin surface soil an average daily amplitude of 53.5° C. was observed in two measurement series (on the afternoon of July 24 and the night of July 25, 1946). The difference between the highest individual maximum and lowest minimum was 57° C. Some cloudiness prevailed during the measurements. Without the clouds a maximum amplitude of over 60° C. would probably have occurred.

The extreme microclimate of this plot was caused primarily by two factors characteristic of this kind of patch in forests: (*a*) these patches are somewhat protected by surrounding saplings from the effects of turbulent mixing; (*b*) the soil is dry because of the action of sapling roots (Table V). In this case the dryness was intensified by the generally low precipitation (Table III).

Moreover, a third, perhaps incidental, factor was involved, namely that of ants tunnelling in the ground. These *Lasius* ants were attracted by the aphids on the saplings and reserve trees in the neighborhood and were very numerous in the middle of the summer of 1946. They made the surface soil loose and effectively insulated it from the stabilizing thermal influence of deeper layers.

In openings of various sizes, Geiger (4) found that the protection given by the surrounding stand caused very high maxima and also relatively high minima. In the present study the gap (plot 3) was also expected to show moderated minima owing to the supposedly decreased radiation. Actually, the reverse temperature effects occurred (Fig. 3), which, aside from the soil condition, were caused by the shape of the gap. This resembled an inverted cone bounded by dense foliage. This shape probably enabled cooler air to flow down from the crowns of surrounding saplings to the bottom of the gap. The gathering cool air was prevented by the foliage from intermixing materially with the warmer air of the surroundings. In openings resulting from cuttings, the situation is different because air can move readily to and from the surrounding stand.

Reflection may also have been a factor of some importance on plot 3. The reflecting ability of green vegetation is considerable (5) and this fact, together with the cone-shaped surface of surrounding green regeneration, may have contributed to the high maximum at this site.

Plot 6 among sparsely stocked saplings showed a relatively high maximum and low minimum (Fig. 7). The plot was somewhat protected from turbulent mixing, as wind measurements (page 766) showed. The soil was fairly compact but subject to the drying action of roots. However, when the measurements for Fig. 7 were made, the soil was still rather moist after rain (Table III). Drought injuries did occur on the plot, but later than on plot 3 (Table V). Sites of this kind obviously have very extreme microclimates.

The plot under the group of pine (plot 4, Fig. 7) was also studied soon after rain, but the surface soil was no longer moist since the crown intercepted a part of the rain. Of the last rainfall before the study, only 41% reached the plot. Places of this kind are commonly drier than the surroundings (1). Often they are also protected from turbulent mixing as well as from outgoing radiation. Thus, the high maximum and the high minimum shown by Fig. 7 can be considered characteristic.

The high maximum found on plot 4 was intensified by the activity of the same kind of ants as on plot 3. On the other hand, the measurements for Fig. 7 were made early, the deeper soil and air still being cool (Tables III and IV). No exact comparison with plot 3 was made and the question remains open whether a gap with, or without, overtopping trees was the hottest forest site.

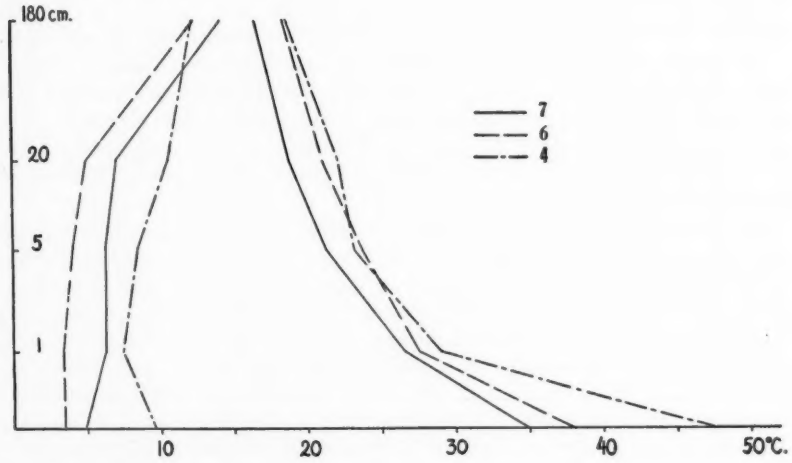

FIG. 7. Late evening (left) and afternoon temperatures at three plots, June 9, 1946. 7 = *Calluna* plot; 4 = under pine group; 6 = in sparse sapling stand; surface soil temperatures measured on denuded sandy spots.

If an overtopping pine is small, its protecting effect is small and the climate under it is intermediate between that of plot 3 and plot 4. But the protecting effect of a larger group of trees is strong and the climate under the southern edge, at least, since it is exposed to direct radiation, generally will be very warm.

The openings in spruce stands (plots 10 and 13, Figs. 2 and 6), being somewhat protected from turbulent mixing, showed high maxima. Minimum temperatures were measured on Aug. 27, 1952, in the spruce stand (plot 12) and in the opening (plot 13). While the minimum thermometer laid on sand showed − 1.0° C. in the opening, + 5.5° C. was recorded in the stand. Similar protection from frost by a canopy is illustrated in Multamaki's study in Finland (9) and studies in Central Europe (5). Other investigations (Geiger (5, p. 351)) have indicated that large gaps have higher maxima than completely open areas. The same observation is illustrated in Fig. 6, which probably represents an extreme case, the soil cover in the gap being thick, porous, and black.

Evaporation at Dry Sites

Evaporation, shown in Table VI, was measured during a dry period favoring strong evaporation (Table III). The daily peaks were measured during a very windy, dry period, July 18 to 19, 1946, when the evaporation in mm./day was:

	Height		
	1 cm.	20 cm.	40 cm.
Calluna plot No. 7	8.4	9.8	10.9
Aerodrome plot No. 5	10.8	12.8	13.3

TABLE VI

EVAPORATION AT VARIOUS SITES DURING THE DRY PERIOD JUNE 25 TO JULY 24, 1946

Plot	Vegetation	Relative evaporation (6.6 mm./day, the rate on plot 7 at 1 cm. = 100)		
		Height		
		1 cm.	20 cm.	40 cm.
5	(Aerodrome)	120	143[1]	149[1]
1	(Burnt area)	117[1]	125[1]	131[1]
7	*Calluna*	100	113	120
4	Under 3 pines	88	—	82
3	Gap in sapling stand	80	—	89
6	Sparse saplings	77	100	99
2	Mature pine stand	55	68	72

[1] *Estimations based on a shorter period of measurement.*

The measurements made by Blomqvist (2) some 100 miles to the south in 1912 and 1913 are comparable with those given above. However, the highest daily evaporation rate found by him at the ground, 6.67 mm., is much less than those given above. He probably did not measure the evaporation during such dry weather and on such a dry open heath area at Siikakangas.

In general, evaporation was the greater the higher the position of the vessels (Table VI). The exception under the group of pines was obviously caused by the high soil temperature (Fig. 7) and the low gradient in wind speed (page 766).

The evaporation vessels at a height of 1 cm. (Table VI) were placed above patches of 40 × 40 cm. which were exposed and denuded to the sand. On plots 6 and 7, vessels were also placed between dense *Calluna* branches. This vegetation caused decreases of 30 and 43% in evaporation.

Similarly a thin layer of pine slash over the vessel reduced evaporation by 69% on plot 7.

The sites exposed to irradiation but still with some protection from wind (plots 3, 4, and 6) showed high temperatures but not high evaporation. Obviously wind was the most important factor favoring high evaporation. Maximum temperatures and evaporation were correlated negatively rather than positively. Since large evaporation vessels were used, temperature variations of the water did not follow closely those of the plots. If evaporation meters of very small dimensions could be used, the results might differ from those obtained.

Discussion

The forest sites studied can be roughly divided into two categories: (*a*) those which are exposed to little radiation and hence have small or moderate daily temperature fluctuation at the ground—these include well-stocked mature stands and sites with dense regeneration or ground vegetation; (*b*) the sites exposed to radiation, such as burnt areas and cut-over areas with little or no vegetation which could protect them from radiation—the thin surface soil of these areas may show very extreme temperatures, at least in patches (Figs. 3 to 7). As was indicated by comparative measurements on pages 764, 765, and 769, the extent of diurnal variation is correlated with the quality of the surface. In compact mineral soils and in all wet soils, temperature extremes are greatly modified.

As already suggested by Vaartaja (13, 14) and confirmed by the present study, maxima as high as 50° to 70° C. are of normal occurrence in exposed surface soils in dry forests while midsummer minima can remain near or below freezing.

The range of diurnal temperature variation is, within certain limits, independent of the general climate. Little correlation existed between temperatures in the surface soil and those in the air at 1.8 meters. The lack of correlation between surface soil temperatures and temperature conditions is explained as follows: the quiet laminar air layer just above the soil

surface and the porous dry soil itself act as insulators and prevent much of the heat produced (or lost at night) by radiation from flowing up and down (or from being compensated for from above or below) (cf. 5, 13).

Various patches in forests, if exposed to radiation, were found to differ in temperature. This was accounted for mainly by the following factors: protection from turbulent mixing by various vegetation; air flows governed by vegetation; protection by crowns from outgoing radiation; soil cover. These are generally considered of importance in microclimates (5). Differences in soil moisture, partly as the result of root activity, were assumed to be important in many cases and may need more attention in future research.

The highest temperatures recorded in the surface soil are injurious to young seedlings, as was shown in the author's experiments not yet published, in Finland, and by German and American investigators (13). Further, in an unpublished test, such daily temperature fluctuations as were found above in extreme cases decreased the germination percentage of seed of *Pinus silvestris*.

Similarly, the results of the seeding experiment carried out on the plots of the present study in 1946 suggest a poor regeneration for the plots with extreme microclimate (14). Though many other factors affected the seeding results, the germination on exposed plots was evidently delayed and reduced by microclimatic factors. Furthermore, the extreme conditions probably killed seedlings and retarded their growth. In cracks in the humus the seedlings were established much better than in the immediate vicinity on fully exposed spots. This is readily explained by the damped microclimate of the cracks illustrated by the measurements shown on page 770. The covering of seeds by litter or sand, either purposefully or through the action of running water, improved the seeding results on exposed plots.

In the United States a high surface soil temperature is commonly considered to be one of the most deleterious factors affecting regeneration. The importance of this factor as a cause of death of young seedlings is recognized by both silviculturists and forest pathologists. A recent study by Smith (11) in Connecticut shows the inferiority of the plots with extreme microclimate in the germination as well as in the early survival of *Pinus strobus*.

Since extreme temperatures may occur even in rather cool climates, as in North Finland in the Arctic Circle (page 770), they are probably widespread in various countries. Prerequisites, however, are a certain amount of strong sunshine, exposure of the site, and a certain dryness.

The results of investigations in Finland concerning the effect of climatic factors on forest regeneration may similarly apply to the greater part of Scandinavia and Canada in burnt and cut-over forest areas. In burnt areas the exposure and black color favor extreme conditions. In many cut-over areas the residual trees and the ground vegetation may serve as a protection from too much exposure, but can, on the other hand, cause patchwise extreme conditions where great exposure and soil dryness, due to root activity, coexist. Actually an occasional thermocouple record of 50° C. in surface sand has been reported under *Pinus banksiana* near Sault Ste. Marie, Ont. (15). In warmer

climates the maxima should be higher owing to the stronger radiation and warmer soil. However, the occurrence of insulating organic layers is rarer owing to rapid decomposition.

The evaporation rate at various sites was not in accordance with the results of Vaartaja's (14) seeding experiment. In fact, drought injuries to *Calluna* and pine saplings occurred mostly where the evaporation rate was low (cf. Tables V and VI). Evidently dense stocking caused low evaporation and, which was more important, also intense root competition and hence drought injuries. The results of seeding experiments (14) also suggested the importance of root competition on the same sites. Furthermore a laboratory experiment, not yet published, proved that conditions of very high evaporation rate had no pathological effect on Scots pine seedlings if the soil was kept moist. The same was established by Daubenmire (3) with six American coniferous species.

However, a high evaporation rate may be somewhat harmful for the germination of seeds and for the growth of seedlings. As the experiments with protection by slash suggested (page 780), there are methods of reducing evaporation markedly without increasing root competition. The various aspects of slash application cannot be discussed here. It should be mentioned, however, that a danger of spreading diseases with slash exists in certain cases.

Summary

1. Comparative thermocouple measurements on clear calm summer days in Finland showed that little difference existed in the temperatures of various forest sites in air at a height of 1.8 meters, whereas the temperature conditions on the same sites differed greatly in the thin surface soil. The conditions at heights of 1, 5, and 20 cm. were intermediate.

2. The exposed sites and the sites protected from radiation showed essentially different microclimates. The sites under dense spruce canopies warmed little by day and mainly by convection from the surroundings, while the less dense pine sites warmed considerably from insolation.

3. The quality of the soil was very important in producing extreme temperatures. Dryness of the soil, especially as intensified by the action of roots, looseness of the soil, and the presence of humus and fine litter favored extreme diurnal variations in temperature.

4. A fluctuation of 57° C. in 24 hr. was recorded in sandy surface soil. Probably a still larger fluctuation occurred in sand, and especially in humus and litter.

5. Maximum surface temperatures of 50° to 70° C. were recorded and are considered of normal occurrence in forest soil at various sites.

6. Temperatures below freezing were often recorded in the middle of summer on exposed forest soil.

7. Protection from wind by ground vegetation or saplings increased the extremity of site conditions. Crowns of a few reserve trees caused warm conditions under them, both day and night.

8. Cracks in humus showed essentially different temperature conditions from surrounding surface humus.

9. Low temperatures (9° to 13° C.) were found under thick vegetation and humus cover, and deeper in the soil in the middle of summer. A negative correlation existed between these temperatures and the thickness of the cover.

10. A negative correlation was found between evaporation rate and drought injuries of seedlings. Root competition was evidently a more important factor to plants than evaporation conditions, at least as shown by measurements from large water vessels.

11. Thin cirrus did not materially affect temperature conditions; the effect of small cumulus was temporary. Wind speed was much retarded near ground level at most sites. Wind affected the temperatures very little. Soil moisture affected especially the temperature variation of surface soil.

Acknowledgments

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THE PHYSIOLOGY OF STOMATA

I. CARBON DIOXIDE FIXATION IN GUARD CELLS[1]

By Michael Shaw and G. A. Maclachlan[2]

Abstract

The guard cells of *Tulipa gesneriana* L. were found to contain both chlorophylls *a* and *b*. The total chlorophyll content was estimated to be of the order of 0.5×10^{-12} gm. per cell. By exposure to radioactive carbon dioxide followed by microautoradiography, selective carbon dioxide uptake in the light was demonstrated in the stomatal cells of *Allium*, *Hordeum*, *Nicotiana*, *Sedum*, *Tradescantia*, *Tulipa*, and *Vicia*. A less intense uptake was observed in darkness with *Allium*, *Sedum*, *Tulipa*, and *Vicia*. The rate of guard-cell photosynthesis was estimated to be of the order of 0.02×10^{-12} M. carbon dioxide per cell per hour at 500 ft-c. and in 0.04% (v/v) carbon dioxide. It was concluded that this rate was too low to account for the increase in osmotic potential caused by illumination.

In 1856 Von Mohl (16) suggested that photosynthesis in the guard cells was the cause of light-induced stomatal opening. It is now clear that light promotes opening mainly through its indirect action in lowering the carbon dioxide tension within the photosynthesizing green leaf; small reductions in the level of atmospheric carbon dioxide cause opening or prevent closure, even in darkness (6, 8, 12). In postulating the possibility of an additional, direct effect of light, Scarth and Shaw (13) suggested that this effect might, in some cases, depend on guard-cell photosynthesis. No evidence was obtained for the direct light effect, nor was the occurrence of stomatal photosynthesis substantiated.

In the past, the presence of what appeared to be chloroplasts led to the general assumption that the stomatal cells were capable of photosynthesis. Despite the reservations of Sayre (11) and the recent statements of Heath (7), there is now virtually no doubt that the pigment in guard-cell plastids, including those of *Allium* species, is indeed chlorophyll. The guard-cell plastids of *Allium* and a number of other species showed the orange–red fluorescence typical of chlorophyll in both ultraviolet (see references in (14)) and transmitted visible light (14); the absorption spectra of individual plastids in living guard cells of *Rumex patientia* (the species employed by Sayre) and of *Chrysanthemum maximum* indicated the presence of both chlorophylls *a* and *b* (17); and finally the extraction of chlorophyll from cleaned epidermal strips of *Hymenocallis littoralis* Salisb. (5) provides additional evidence, though only if the common epidermal cells of this species lack chloroplasts.

The presence of chlorophyll is not in itself proof that photosynthesis occurs. Freeland (5) made a direct attempt to detect guard-cell photosynthesis using cultures of *Photobacterium fischeri*, which glows in the presence of minute

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Contribution from the Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan.

[2] *Professor of Biology (Plant Physiology) and graduate student, respectively.*

traces of oxygen. The results were negative but were considered inconclusive. Recently Dyar (3) showed that the guard-cell plastids of spinach, like those of the mesophyll, reduced "blue tetrazolium" under anaerobic conditions. Reduction was confined to the grana. If the suggestion that the dye functions as an oxidant in the Hill reaction be correct, Dyar's results may be cited in favor of guard-cell photosynthesis.

Alvim (1) and Scarth and Coulombe (see (13)) considered that the formation of starch in guard cells, previously starved until starch-free and then illuminated in the presence of carbon dioxide, demonstrated the occurrence of photosynthesis. Alvim worked with isolated epidermal strips of *Nicotiana*, *Phaseolus*, and *Zebrina* and obtained his best results with *Nicotiana tabacum*. The epidermal cells of many dicotyledonous species possess chloroplasts, though these vary in size, intensity of pigmentation, and ease of detection. In the common epidermal cells of *Nicotiana tabacum* the chloroplasts are conspicuous. Thus, in Alvim's experiments starch-formation in the guard cells may equally well have resulted from the entry of soluble carbohydrates from surrounding cells. The results of Scarth and Coulombe, who worked with the albino portions of variegated *Pelargonium*, are also open to question on these grounds. The senior author has found traces of chlorophyll in the albino portions of leaves of this species.

It must therefore be concluded that, despite the presence of chlorophyll and the effectiveness of carbon dioxide tension in influencing stomatal movement, there is no unequivocal evidence in the literature to indicate that carbon dioxide fixation, photosynthetic or otherwise, actually occurs in stomatal guard cells.

The experiments to be described were undertaken in an attempt to settle this important point of stomatal physiology by exposing isolated epidermis to radioactive carbon dioxide followed by microautoradiography. A preliminary account of this work was published in *Nature* (15).

Material

Tulipa gesneriana L., *Hordeum vulgare* L., *Tradescantia zebrina* Hort., and *Allium cepa* L. provided epidermis in which only the stomatal guard cells possessed chloroplasts. In tulip 75–90% of both guard and epidermal cells, in barley and *Tradescantia* only the subsidiary and guard cells, and in *Allium* only the guard cells survived the preparative and experimental treatment.

Both the guard and epidermal cells of *Nicotiana tabacum* L. and *Vicia faba* L. possess chloroplasts. Twenty to 70% of the epidermal and 90% of the guard cells survived treatment. "Epidermis" stripped from *Sedum praealtum* D.C. was also employed. This consisted of two layers of cells, the lower being chlorophyllous.

In all cases cell survival was estimated by neutral red staining. The presence or absence of chloroplasts was determined by close inspection of living cells at high magnification and by the use of 1% silver nitrate at pH 4.0 (14).

Individual strips of tulip epidermis measured 0.5–1 × 4–8 cm. These were strong enough to withstand vigorous scraping, and 1–3 gm. (fresh weight) samples were readily obtainable. This tissue was therefore selected for approximate quantitative determinations of chlorophyll content and carbon dioxide fixation. The epidermis of the remaining species was used for microautoradiography only.

Methods

(*1*) *Preparation of Tulip Epidermis*

Strips were removed from the leaves with forceps and placed in tap water. Each strip was carefully scraped with a razor blade and the margins trimmed. Then they were washed for two hours in several changes of water and inspected under the dissecting microscope. A similar technique was used by Freeland (5). Where quantitative determinations were to be made, those strips from which all adhering mesophyll did not appear to have been removed were discarded. Fresh weight (2.68 ± 0.252 mgm./cm.^{2}) was determined by stripping the entire epidermis from 20–50 1-cm.^{2} squares of leaf obtained with a specially constructed cutter. The epidermis was scraped, washed, and blotted dry with filter paper before being weighed. Dry weight (0.55 ± 0.075 mgm./cm.^{2}) was determined after 24 hr. at 80° C. in a forced air oven. Stomatal frequency (3600 ± 200/cm.^{2}) was determined on fresh strips mounted in water. Guard-cell volume (6.5×10^{-9} cm.^{3}) was determined from camera lucida drawings of stomata in cross section and surface view, using fresh material mounted in water.

(*2*) *Chlorophyll Extraction and Estimation*

A known fresh weight of tissue was thoroughly ground in a mortar or blender with 85% acetone and ether until colorless. A small quantity of calcium carbonate was mixed with the tissue before it was ground. The ether layer of the combined extracts was concentrated, dried by the addition of anhydrous sodium sulphate, and made up to a known volume with anhydrous ether. These operations were carried out in a darkened room.

Absorption spectra were determined with a Beckman (Model DU) spectrophotometer, using 1 cm. cells. The concentrations of chlorophylls *a* and *b* were calculated using the equations of Comar and Zscheile (2).

(*3*) *Exposure to Radioactive Carbon Dioxide*

This was carried out in two large glass jars (*ca.* 18 liters), one of which was light proofed for dark exposures. The pressure in each jar was reduced by 20 m. of water and the required volume of radioactive sodium carbonate solution then injected into perchloric acid in an attached 10 ml. generating flask. The latter was fitted with a tube and screw clamp so arranged that when the clamp was released a stream of air bubbled through the carbonate – perchloric acid mixture, thus sweeping the radioactive carbon dioxide into the jar.

The system was sealed again by closing the clamp as soon as bubbling ceased. The total amount of carbon dioxide introduced by the above procedure in the standard experiments was 0.22 ml. (at s.t.p.), a quantity insufficient to alter appreciably the 'normal' carbon dioxide content of the atmosphere (0.03% v/v).

During exposures, which were always of three hours' duration, the epidermal strips were floated, cuticle uppermost, on water in Petri dishes. In all 'dark' experiments the tissue was kept in darkness for approximately one hour before introduction of the tracer in order to eliminate the effect of 'reducing power' generated by previous illumination, and the jar was opened, after flushing, in a dark room and the epidermis killed without exposure to light. Normally the strips were washed briefly with 2% hydrochloric acid to remove adsorbed radioactive carbon dioxide and then dipped in absolute alcohol before being dried flat between sheets of paper or dried directly on microscope slides. The omission of washing did not affect the results obtained by microautoradiography.

(4) *Microautoradiography*

The best results were obtained with Ilford G. 5 emulsion. This was purchased from Ilford, Ltd., Ilford, England, in the form of plates (1 in. × 3 in.) with an emulsion 5μ thick protected by a 1μ layer of gelatin and also as a free gel. Accordingly, two techniques were employed. In the first, or 'contact' technique, microscope slides bearing radioactive epidermis were placed in contact with dry Ilford plates. In the second, or 'pouring' technique, a thin layer of the free emulsion, melted at 40° C., was painted onto epidermis samples mounted on slides. The emulsion hardened immediately upon cooling. After three to six weeks' exposure in darkness at 4°–5° C. both the contact and poured plates were developed for 20 min. in Ilford D19 developer, washed in 2% acetic acid, and fixed until clear. After washing and drying, the plates were ready for microscopy. A few radiographs were also made using X-ray film. A general review of autoradiographic techniques has been published by Fitzgerald *et al.* (4).

Results

1. Chlorophyll Content of Tulip Epidermis

The absorption spectra of ether extracts of epidermis and whole leaf of tulip respectively are shown in Fig. 1. Both curves show the absorption maxima characteristic of chlorophyll and are closely similar. The presence of both chlorophylls *a* and *b* in the epidermal extracts was confirmed by paper chromatography in water-saturated butyl ether. With this solvent excellent separation of chlorophylls *a* and *b* and the xanthophylls and carotenes was achieved in three hours at 20°–22° C. The concentrations of chlorophyll per gram fresh weight and per guard cell are given in Table I. In the 1953 experiment the ratio of chlorophyll *a* : chlorophyll *b* for the epidermis was

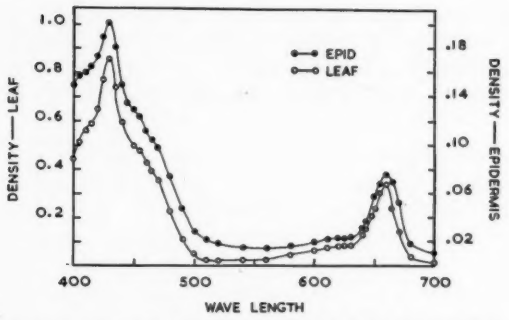


FIG. 1. Absorption spectra of ether extracts of epidermis (solid points) and whole leaf (open circles) of tulip respectively. Ordinate: $\log_{10} I_0/I$. Abscissa: wave length in millimicrons.

1.81 while that for the whole leaf was 3.45. This large difference in ratio was not observed in the 1954 experiment but the authors believe both sets of results to be valid. The 1953 analysis was carried out on post-flowering plants; in 1954 the plants had not yet flowered. The chlorophyll content per guard cell was estimated to be 1.41 and 0.54 $\times$ 10^{-12} gm. in 1953 and 1954 respectively. Since the main source of error in these determinations consists in the possible failure to remove traces of mesophyll from the epidermal strips, the authors prefer to accept the lower estimate as an indication of the probable order of magnitude of chlorophyll content.

TABLE I

CHLOROPHYLL ESTIMATIONS

Experiment	Chlorophyll	Epidermis				Leaf			
		a	*b*	*a* + *b*	*a*/*b*	*a*	*b*	*a* + *b*	*a*/*b*
June 22, 1953	μgm./gm. fresh wt.	2.47	1.36	3.83	1.81	283	82	365	3.45
	gm. $\times$ 10^{-12} /guard cell*	0.91	0.50	1.41					
June 10, 1954	μgm./gm. fresh wt.	0.93	0.53	1.46	1.73	496	267	763	1.85
	gm. $\times$ 10^{-12} /guard cell*	0.35	0.19	0.54					
June 8, 1954	μgm./gm. fresh wt.					648	349	997	1.85

* *Calculated from a stomatal frequency of 3600 per cm.² and a fresh weight of 2.68 mgm. per cm.² epidermis. In the preliminary account (15) of this work the results for June 1953 were calculated from a fresh weight of 5.3 mgm. per cm².*

2. Carbon Dioxide Fixation

A. Microautoradiography

Small samples of isolated epidermis were exposed to radioactive carbon dioxide for three hours (total carbon dioxide concentration 0.03% approximately; specific activity of carbon dioxide in gas phase 0.24%) in the diffuse light of the greenhouse and in darkness respectively. After exposure the strips were washed, mounted, and radiographed.

The results are illustrated by the photomicrographs of autoradiographs in Plates I and II. Fig. 2 is a 'light' strip of tulip epidermis radiographed on X-ray film. Figs. 3, 4, 5, and 6 illustrate the results obtained in light exposures of bean and tobacco by the 'pouring' technique. Figs. 5 and 6 show particularly clearly the correspondence between radioactivity and the starch bearing chloroplasts, which are stained with iodine in Fig. 6. Figs. 8 and 9 show, respectively, 'light' and 'dark' uptake in the stomatal cells of *Allium*, and a single stoma from a light strip is shown in Fig. 10. These results with *Allium* were obtained by use of the 'contact' technique. Figs. 7, 11, 12, and 13 show, respectively, radiographs of single stomata from 'light' strips of bean, barley, *Sedum*, and *Tradescantia*, also obtained by the contact technique.

'Dark' uptake in the guard cells was occasionally more pronounced than indicated by Fig. 9 but was clearly not as intense as that in the light. Greater activity in the guard than in surrounding cells as a result of dark uptake was also observed for bean, *Sedum*, and tulip. With tobacco, in which the epidermal cells possess very conspicuous chloroplasts, there was little or no difference in the intensity of radioactivity associated with the epidermal and stomatal cells following dark exposures. The failure to demonstrate dark uptake in guard cells of bean and onion reported earlier (15) was due to imperfect contact between emulsion and tissue during radiography.

Strips not exposed to radioactive carbon dioxide and strips exposed after killing did not fog the emulsion.

The simplest interpretation of these results is that photosynthesis occurs in the guard cells. Fixation of carbon dioxide by 'dark' reactions occurred in both guard and surrounding epidermal cells. The validity of these conclusions is discussed later.

B. Quantitative Comparison of 'Light' and 'Dark' Fixation by Tulip Epidermis

The object of these experiments was to determine an order of magnitude for the rate of guard-cell photosynthesis to enable assessment of its role in contributing to changes in osmotic potential.

Two samples of tulip epidermis, each of approximately one gram fresh weight, were carefully cleaned and exposed to radioactive carbon dioxide in light and darkness respectively at a temperature of 27° ± 1° C. for three hours, following the procedure described earlier. After exposure, the samples were washed and oven dried at 80° C. for 24 hr. Individual strips were autoradiographed (see Fig. 2) and the remainder of each sample ground to

pass a 60-mesh sieve. The activities in counts per minute were then determined at infinite thickness with a gas flow counter (Proportional Counter, Model PC-1, Nuclear Measurements Corp.). These results were then converted to microcuries per gram dry weight by the use of the appropriate radioactive standards.

The experiment was carried out first in June, 1953, using field-grown tulips (Experiment I) and again during January, 1954, using greenhouse-grown plants (Experiment II). In Experiment I the amount of radioactive plus carrier carbon dioxide (0.22 ml. at s.t.p.) added to each chamber (volume of dry air at s.t.p. = 17.5 liters) was insufficient to alter appreciably the normal carbon dioxide content of the air. In Experiment II 3.40 ml. (at s.t.p.) were added, and the light intensity was also raised from 300 to 700 ft-c. in an effort to increase photosynthesis. The experimental conditions and results are given in Table II.

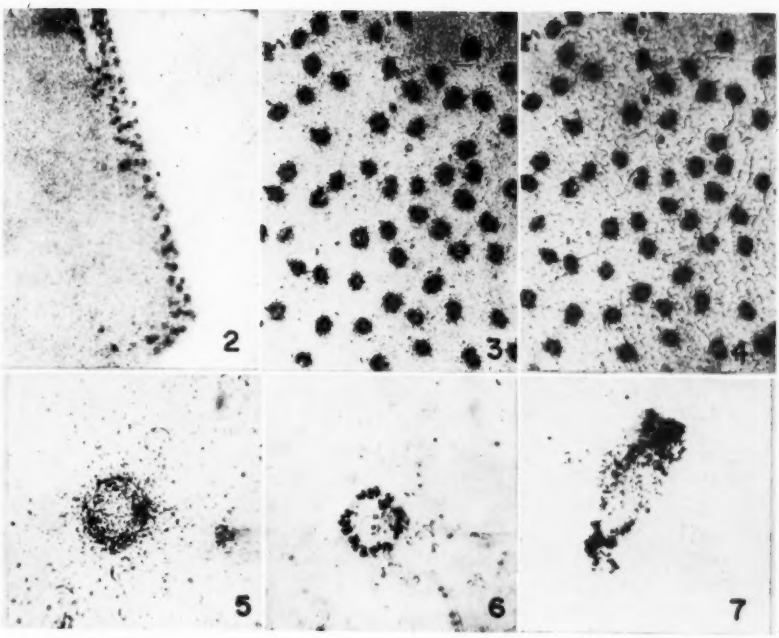
TABLE II

CARBON DIOXIDE FIXATION

	Experiment I (June, 1953)				Experiment II (January, 1954)			
	Light (300 ft-c.)		Dark		Light (700 ft-c.)		Dark	
Assumed initial volume CO_2 per chamber (% v/v)	0.030	0.040	0.030	0.040	0.030	0.040	0.030	0.040
Final volume CO_2 per chamber (% v/v)	0.0313	0.0413	0.0313	0.0413	0.0495	0.0595	0.0495	0.0595
Initial specific activity of CO_2 in gas phase (% v/v)	0.24	0.18	0.24	0.18	0.16	0.13	0.16	0.13
Activity (counts/min.)	1016		578		1139		331	
Activity (μc./gm. dry wt.)	0.118		0.067		0.135		0.039	
CO_2 fixation: (moles $\times 10^{-8}$/gm. dry wt./3 hr.)	0.99	1.30	0.56	0.74	1.74	2.09	0.51	0.61
CO_2 fixation: (moles $\times 10^{-12}$/guard cell/3 hr.)	0.032	0.041			0.091	0.109		
Equivalent osmotic inc. (atm./cell/3 hr.)	0.10	0.13			0.28	0.34		

From the initial specific activity of the carbon dioxide in the chambers and the activity of the samples it was possible to calculate approximately the amount of carbon dioxide fixed per gram dry weight of tissue. The specific activity of the carbon dioxide in the chamber decreased during the experimental period since the tissue was shown by conventional manometric techniques to respire 50 to 60 μl. of carbon dioxide per gram fresh weight per hour, while the calculated fixation in light was only 2–3 μl. per gram fresh weight per hour. The error thus introduced is small (2–3%) and has been neglected. Though the carbon dioxide concentration of the air before the addition of the tracer was close to 0.03% v/v, it was not actually measured.

PLATE 1



FIGS. 2-7. Experimental treatment: three hours in radioactive carbon dioxide in light. FIG. 2. Radiograph of tulip epidermis on Kodak X-ray film. Radioactivity is localized in the guard cells (*ca.* × 15). FIG. 3. Radiograph of bean epidermis obtained by the 'pouring' technique (*ca.* × 60). FIG. 4. Same field as in Fig. 3 but focused at the level of the tissue (*ca.* × 60). FIG. 5. Radiograph of tobacco stoma obtained by the 'pouring' technique (*ca.* × 270). FIG. 6. Same stoma as in Fig. 5, but focused at the level of the tissue to show starch grains stained with aqueous iodine (*ca.* × 270). FIG. 7. Radiograph of bean stoma obtained by the 'contact' technique (*ca.* × 580).

PLATE II

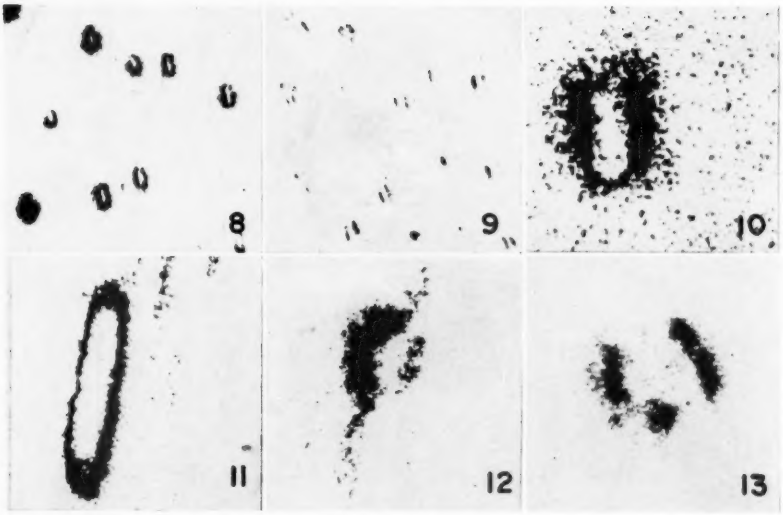


FIG. 8. Radiograph of onion epidermis obtained by the contact technique. After three hours in radioactive carbon dioxide in light (*ca.* × 120). FIG. 9. Radiograph of onion epidermis obtained by the contact technique. After three hours in radioactive carbon dioxide in darkness (*ca.* × 120). FIGS. 10, 11, 12, 13. Radiographs of single stomata of onion, barley, *Sedum*, and *Tradescantia*, respectively. Obtained by the 'contact' technique after three hours in radioactive carbon dioxide in light (*ca.* × 580).

Carbon dioxide fixation was therefore calculated from specific activities corresponding to initial carbon dioxide concentrations of 0.03 and 0.04%.

Autoradiographs had revealed that the radioactivity per unit area of emulsion was greater at guard-cell positions than elsewhere on the epidermis in both 'light' and 'dark' treatments. Moreover, guard-cell activity in the light was greater than that in the dark. It was therefore decided to adopt the difference between light and dark fixation per gram dry weight as a measure of guard-cell photosynthesis. This is legitimate since only the guard cells of tulip epidermis contain chloroplasts. Photosynthesis (as carbon dioxide uptake per guard cell) was then calculated from the stomatal frequency and the wet and dry weights of the tissue per $cm.^2$. The vacuolar volume of a 'closed' guard cell was taken for purposes of calculation as 20% of the cell volume, i.e. 1.3×10^{-9} $cm.^3$. On the assumption that all the carbon fixed was converted to hexose, it was possible to calculate the equivalent osmotic rise per guard cell caused by illumination, the osmotic potential of a 1 *M* solution of glucose being taken as 24 atm.

The results of all these calculations are given in Table II, and the main conclusions may be summarized as follows:

(i) Fixation in the dark was similar (*ca.* 0.53×10^{-6} M. carbon dioxide per gm. dry weight per three hours) in the two experiments, despite the higher carbon dioxide concentration in Experiment II.

(ii) Fixation in the light (0.99 and 1.74×10^{-6} M. carbon dioxide per gm. dry weight per three hours) was approximately two and three times that in the dark in Experiments I and II respectively. Light fixation was approximately doubled by increasing the light intensity from 300 to 700 ft-c. and the total carbon dioxide concentration from 0.03 to 0.05%. It is considered that this effect is too great to have been caused by a difference in the concentration of atmospheric carbon dioxide on the two occasions. Any such difference is unlikely to have exceeded 0.003% v/v. The mean rate of photosynthesis per guard cell in the two experiments was $0.021–0.025 \times 10^{-12}$ M. carbon dioxide per hour. Owing to an error in calculation the rate reported earlier (15) is not correct.

(iii) On the basis of the assumptions made in the calculations and under the conditions of these experiments, the rate of fixation per guard cell in the light corresponds to an increase in osmotic potential of 0.10 to 0.34 atm. in three hours.

Discussion

The results of the chlorophyll estimations indicate that the guard cells of tulip contain both chlorophylls *a* and *b*. This conclusion is in agreement with the results of Yemm and Willis (17), obtained by microspectrophotometric measurements on single guard-cell and mesophyll chloroplasts. Freeland (5) reported that the pigment in the stomatal cells of *Hymenocallis littoralis* Salisb. was mainly chlorophyll *a*.

According to Rabinowitch (10) Godnev and Kalishevich estimated the chlorophyll content of a single chloroplast of *Mnium* to be 2.4×10^{-12} gm. The volume of an average chloroplast was 4.1×10^{-11} ml. and the concentration of chlorophyll in it was therefore 0.06 gm. per ml. Taking the chlorophyll content of a single guard cell as 0.54×10^{-12} gm. and the average number of chloroplasts per cell as 15, a single guard-cell chloroplast of tulip contains 0.036×10^{-12} gm. of chlorophyll. A guard-cell chloroplast measures approximately 1.5×3 μ and its volume is close to 1×10^{-11} ml. The concentration of chlorophyll in it is therefore approximately 0.0036 gm. per ml. or roughly 1/16 the concentration in a mesophyll chloroplast of *Mnium*. This estimate could, presumably, be checked by microspectrophotometry.

The simplest explanation of the results obtained by microautoradiography is the occurrence of photosynthesis in the guard cells. The tissue was, however, exposed to radioactive carbon dioxide for three hours and the possibility of the redistribution of fixed carbon cannot be ignored. It has been suggested elsewhere (13) that light-induced opening is due, in part, to the polar transport of osmotically active solutes into the guard from surrounding cells. Thus there was ample time for such movement to occur and it may well have contributed to the results with bean, tobacco, tulip, and *Sedum* in which a proportion of the nonstomatal cells remained alive. From the distinctness of the guard-cell radiographs it appears that this is not the sole mechanism of uptake. Moreover, such movement cannot have occurred in barley and onion. In the former only the guard and subsidiary cells survived stripping and activity appeared to be localized in the guard cells (Fig. 9); in the latter only the guard cells survived and activity was strictly localized in these.

With respect to the quantitative estimates of carbon dioxide fixation it is regarded as unlikely that the enhanced fixation obtained in light was caused by nonstomatal photosynthesis. Any mesophyll cells not completely removed by the preparative treatment were certainly broken and the chloroplasts being freely exposed to water must quickly have swollen and burst. The radiographs of individual strips (see Figs. 2 and 3) did not reveal the presence of the relatively large areas of high radioactivity which result from the presence of photosynthesizing mesophyll cells.

Taking the chlorophyll content of a guard cell as 0.54×10^{-12} gm. and the rate of photosynthesis per cell as the mean value of the estimates at an initial carbon dioxide tension of 0.03% in Experiments I and II respectively, viz.: 0.021×10^{-12} M. carbon dioxide per hour, the number of grams of carbon dioxide fixed per gram of chlorophyll per hour is 1.7. Willstatter and Stoll (see (10)) found that from 5 to 15 gm. of carbon dioxide were fixed per gram of chlorophyll per hour in the leaves of a number of land plants under normal conditions. Comparison of these figures suggests that a five- to ten-fold increase in the rate of photosynthesis in the guard cells is entirely possible under normal conditions.

Sayre (11) gave the difference between the osmotic potential of open and closed stomata as about six atmospheres. We have found a difference of

the order of 5–10 atm. for onion, *Pelargonium*, bean, and tulip. Under optimum conditions full opening may occur in as short a time as one hour. The rate of increase in osmotic potential as measured by the plasmolytic method may therefore be taken as lying between 2 and 10 atm. per hour during rapid opening. The data in Table II suggest that the direct contribution of guard-cell photosynthesis could account for an increase of only 0.03–0.1 atm. per hour. A tenfold increase in the rate of photosynthesis would raise this estimate to 0.3–1.0 atm. per hour. This represents a significant contribution to the osmotic change, particularly at the lower rates of increase, but the contribution of photosynthesis to osmotic rise must be regarded as an overestimate in view of the assumptions made in the calculations (see above). Thus it is probable that the maximum rate of guard-cell photosynthesis is of the order of 50 times too slow to account for the maximum observed rates of change in osmotic potential. On the other hand, the cumulative effects of continued photosynthesis must play a definite, though minor, role in contributing to the osmotic increase, and this may constitute *one* direct effect of light on the stomata. Guard-cell photosynthesis must also contribute to the lowering of the carbon dioxide tension at guard-cell centers, which is accomplished through mesophyll photosynthesis. Since opening is promoted by low carbon dioxide tensions even in darkness, removal of free carbon dioxide or at least the maintenance of a low tension of free carbon dioxide appears to be more important than the direct photosynthesis of osmotically active solutes in the guard cells.

It is pertinent to note here that Heath and Russell (9) have recently obtained results which they interpret as suggesting the occurrence of a light effect which is independent of carbon dioxide. They point out that it is not clear whether this effect is due to a response in the guard cells or to the transmission of a stimulus from epidermal or mesophyll cells. While there is no theoretical objection to this interesting possibility, it is also not clear that the phenomenon is not a readjustment in stomatal aperture following turgor changes in the mesophyll and epidermal cells of the experimental area of the leaf.

Acknowledgments

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TRANSLOCATION PATH AND PLACE OF ACTION OF 3-(4-CHLOROPHENYL)-1:1-DIMETHYLUREA IN BEAN AND TOMATO[1]

By Wm. Harold Minshall[2]

Abstract

Except for a short time lag in the appearance of symptoms, a graded concentration series of CMU in water produced similar effects on intact plants and on excised tops of tomato and kidney-bean. When applied to roots, CMU had ready access to the vascular system and was quickly carried in the xylem to the tops. Wherever a lethal concentration was reached, the CMU killed the tissues of the leaves. As roots of treated plants lived for some days after the leaves were killed, the foremost toxic action of CMU must have taken place in the leaves.

Introduction

The compound 3-(4-chlorophenyl)-1:1-dimethylurea (CMU) has been tested in the field as a pre-emergent herbicide and as a soil sterilant. As yet, however, little has been published on the path of movement of CMU within plants or on its mode of action on plants. Bucha and Todd (1) reported that CMU acts readily through the root system and is translocated upwards to the leaves. Haun and Peterson (2), in a paper read by D. Wolf before the First National Weed Control Conference in December, 1953, at Kansas City, Missouri, stated that, when radioactive CMU was applied in nutrient solution to tomato, the CMU could be detected in the tops of plants within two hours and continued to accumulate in the tops until they were killed some days later.

In studies preliminary to investigations on the mode of action of CMU it was noticed that the first leaves to develop symptoms were those exposed to conditions conducive to a high rate of transpiration. This observation suggested that the CMU was moving in the xylem with the water stream. An account is hereby given of investigations on the movement of CMU and its place of action in plants.

The Effect of CMU on Excised Tops and Intact Plants

Experimental

Excised tops and intact potted plants of 5-week-old tomato (*Lycopersicon esculentum* Mill, var. Bonny Best) and kidney-bean (*Phaseolus vulgaris* L., var. Black Valentine) were exposed to a graded series of concentrations of CMU in water. The tests were carried out in the greenhouse in January. Potted plants were watered from the bottom with the solutions; cut ends of the excised tops were immersed in the solutions.

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[2] *Senior Plant Physiologist.*

Excised tops were employed in these uptake studies to permit direct access of the solution to the xylem of the plant; but as it has been shown (3) that stomatal movement and transpiration rate of excised stems of alfalfa and potato differ from those of potted plants, the use of excised tops may be questioned. Rate of water loss was determined with excised tops of tomato and bean under conditions of continuous illumination from a 500-watt incandescent lamp, at a temperature of 84° F. and a relative humidity of 25%. For two or three hours after cutting, the excised tops continued to take up and transpire water at approximately the same rate as did the intact plant. Following this time, however, the rate of water loss decreased slowly until, by 24 hr. after cutting, it was approximately half the rate of that in the intact plant and, by 48 hr., approximately one quarter. Excised tops of tomato and bean, therefore, took up and transpired a moderate amount of water. They continued to do so until they formed adventitious roots on their stems. In water all excised tops remained turgid and maintained a normal green color; beans continued to flower and formed pods. Excised tops of tomato and bean, therefore, were considered satisfactory material for the present purposes.

Results

Small water-soaked spots soon developed on the leaves of all tomato plants treated with CMU. With time these spots turned to brown necrotic blotches. After the development of blotches the leaves gradually became flaccid and then slowly withered and died. In comparison the water controls continued normal in appearance for the duration of the test.

Excised tomato tops standing in concentrations of 32, 8, and 2 p.p.m. of CMU developed water-soaked spots in two, two, and three days, respectively. Intact potted tomato plants watered with these concentrations required three, four, and five days for spots to appear. This one- or two-day time lag in the appearance of symptoms on the intact plants was maintained throughout the development of the wilt and up to the eventual death of the tops. Except for the difference in time of development of symptoms, however, the effect of CMU on tops of tomato plants was identical in excised and in intact tops.

On the 15th day, when the tomato leaves were dead, most of the roots of intact potted plants were normal in appearance, absorbed the vital stain neutral red, and their cells plasmolyzed in hypertonic calcium chloride.

Alhough in beans, as in tomato, the first water-soaked blotches appeared on leaves of excised plants one day earlier than they appeared on leaves of intact plants, the effect of CMU on beans differed somewhat from its effect on tomatoes. From four to five days were required for water-soaked blotches to appear on the bean leaves; in addition, the development of the symptoms differed in other respects, the description of which is beyond the scope of this paper and will be dealt with elsewhere. But the effect of the CMU on the intact and the excised tops of bean was identical and the water control bean plants were normal in appearance for the duration of the test.

FIG. 1. The protective effect of darkening a primary bean leaf while the excised top was absorbing CMU. Stem of excised top immersed for 21 hr. in 128 p.p.m. CMU on Feb. 3. Photographed Feb. 10.

The Effect of Darkening a Leaf on the Expression of CMU Symptoms

Experimental

Bean plants four weeks of age with the tops consisting of the pair of primary leaves and two trifoliate leaves were placed in the dark until their stomata were closed. An envelope made of black paper was placed over one of the primary leaves and closed around the petiole so as to exclude light from the blade of the leaf. The bean plant was then cut off close to the level of the soil and the lower part of the stem of the excised top was immersed in a solution of 128 p.p.m. CMU. During the absorption period and for the remainder of the experiment the plants were kept at a temperature of 80° F. and received 500 ft-c. of continuous light from a 500-watt incandescent lamp. At the end of 21 hr. the solution of CMU was replaced with water and after a further period of eight hours the paper envelope was removed from the covered leaf.

Results

The leaves of beans that were exposed to light while the plant was absorbing CMU developed blotches typical of CMU injury on the second day and were dried up and dead by the sixth day. In contrast, those leaves that were covered while the plants were exposed to CMU were normal in all respects for at least six or seven days. (Fig. 1).

Discussion

When the tops of plants are severed from the roots the uptake of liquid by the tops is unaffected by any restrictive barrier action on the part of the membranes of the root cells. Until such time, therefore, as callus forms on the cut end of an excised stem, the solution in which the stem is standing enters the open ends of xylem vessels and is carried to the leaves in lieu of the xylem sap. After being severed, both the control and treated excised tops remained turgid. All excised tops apparently took up sufficient water to supply the requirements of the tops, including the transpiration requirement. In those excised plants standing in CMU the loss of water vapor from the leaves and the replacement of this water by the CMU solution by way of the vascular system would bring about a gradual accumulation of CMU within the tissues of the leaves. Wherever a lethal concentration of CMU was reached within the leaf, cells were killed and the cell contents diffused out, resulting in water-soaked areas. These areas then gradually became brown necrotic blotches typical of CMU injury under the environmental conditions of these experiments.

Except for a short time lag in the appearance of symptoms, intact plants behaved in a manner similar to excised tops. It is evident, therefore, that in bean and tomato CMU was readily taken up by the root system and conducted up to the leaves in the water stream. Because of transpiration, it accumulated in the leaves, produced brown necrotic blotches, and brought about eventual death of the tops.

Conclusive evidence that CMU moved in the xylem was afforded by the darkened leaf experiment. Covering the bean leaf to decrease transpiration, and thus reducing to a minimum the amount of CMU entering the darkened leaf, protected the leaf from the typical localized blotched effect caused by this chemical.

As roots of tomato, when exposed to 32 p.p.m. or less of CMU, remained alive until after the leaves were killed, the CMU must not have accumulated to a lethal concentration in the roots—at least not while it was being transported to the tops. Haun and Peterson (2) observed that while radioactive CMU accumulated very gradually in the roots of tomato, it accumulated quite rapidly in the tops. Apparently the foremost toxic action of CMU occurs in the tops of plants and especially in those leaves with a high rate of transpiration.

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DECAY IN POPLAR PULPWOOD IN STORAGE[1]

By Clara W. Fritz[2]

Abstract

Deterioration of poplar pulpwood (a mixture of *Populus tremuloides* Michx. and *P. balsamifera* L.) on Manitoulin Island, Ontario, was studied in field, pit, and block pile storage with reference to the reaction of the rot fungi present in the living tree to storage conditions, and the penetration of fungi which attack wood subsequent to felling. The rots found most prevalent in the living trees, and the most important from the standpoint of volume of wood affected, were the white trunk rots caused by *Fomes igniarius* var. *populinus* (Neu.) Campb., *Radulum casearium* (Morgan) Lloyd, *Polyporus dryophilus* var. *vulpinus* (Fr.) Overh., a rot associated with a non-*Basidiomycete* designated in this paper as *Fungus X*, and the brown rot produced by *Poria cocos* (Schw.) Wolf. The first four rots may survive in storage, but are not appreciably active. Butt rots caused by *Armillaria mellea* (Vahl.) Quél., *Pholiota spectabilis* (Fr.) Quél., and a gray–brown butt rot were not uncommon, but of limited importance both on account of the amount of wood attacked and the practical inactivity of the causal agents in storage. The only tree-rot fungus found likely to cause damage in storage was *P. cocos;* it produces brown rot and is capable of spreading vigorously in stored wood. Other tree rots occurred, but were of minor importance. *Stereum purpureum* Pers. was the first fungus to infect the wood after the trees were felled; it spread rapidly and fruited during the first summer. It was followed by three species of *Polyporus, P. adustus* (Willd) Fr., *P. hirsutus* (Wulf.) Fr., and *P. versicolor* (L.) Fr. These became established in the wood during the first summer; they fruited on field and pit logs during the second season, and were fruiting luxuriantly on block pile logs which had been stored three years. They produce white rots and in some logs caused advanced decay in two years' storage. Other storage rot and staining fungi were isolated from the bolts under study, but during the period of observation did not effect widespread penetration. The same pattern of decay and succession of fungi were found in all types of storage investigated.

Poplar is being used in increasing amounts in the production of both groundwood and chemical pulp. Unfortunately poplar wood, both in the living tree and in storage, is attacked by many fungi which cause discoloration and decay. Newsprint containing poplar pulp made from defective wood is often poor in color, sometimes so dark that the paper is not acceptable; in addition, decay may cause reduction in pulp yield. Losses may not be so extensive in the production of chemical pulp, because many of the fungi which attack poplar cause white rots, in the early stages of which degradation of cellulose is not serious; but in the case of groundwood the weakening of the fiber may lead to marked reduction.

In order that a mill may have a continuous supply of wood it is necessary to establish storage piles, and in these, the bottom logs may lie for two or more seasons; sometimes also, owing to inadequate shipping facilities or other difficulties, wood may be held over in the woods or at loading points for a year or longer. Since poplar has only recently become economically important, no detailed information is available about defects in the wood.

The present paper discusses the fungi found active in poplar wood under study from the autumn of 1950 until February, 1954. All the wood was cut

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[2] *Formerly Timber Pathologist, Forest Products Laboratory, Ottawa, Canada. Private communication.*

on Manitoulin Island, Ont., Canada; two species were included, *Populus tremuloides* Michx. and *P. balsamifera* L., which were mixed without distinction at the felling sites. The material included: (1) samples from freshly felled trees, which were studied to establish the rots which might enter storage piles from the living tree; (2) bolts stored in covered pits below ground level for one and two summer seasons; (3) bolts stored in corded piles for one and two summer seasons; and (4) bolts stored for three years in a mill block pile. The field and pit piles contained as many of the tree rots as were available when the piles were set up.

Rots From the Living Tree

During the summer of 1951 an officer of the Division of Forest Biology, Canada Department of Agriculture, who was investigating the rots in poplar trees on Manitoulin Island supplied the writer with 172 samples of poplar containing different types of rot; these had been cut from 147 trees as soon as they were felled. From these samples some 2300 cultures, developed on malt agar, were studied; these revealed the presence of the following tree-rot fungi:

Armillaria mellea (Vahl.) Quél.,
Corticium polygonium Pers.,
Fomes igniarius var. *populinus* (Neu.) Campb.,
Pholiota adiposa Fr.,
Pholiota destruens Brond.,
Pholiota spectabilis (Fr.) Quél.,
Pleurotus subareolatus Peck,
Polyporus dryophilus var. *vulpinus* (Fr.) Overh.,
Poria cocos (Schw.) Wolf.,
Radulum casearium (Morgan) Lloyd,
Stereum murrayi (Berk. and Curt.) Burt.

A Basidiomycete associated with, and probably responsible for, a gray–brown butt rot was also isolated, but was not identified. This fungus, both in culture and in naturally decayed wood, produced mycelial strands composed of brownish, septated hyphae in parallel formation; clamp connections were noted, but were infrequent.

A non-Basidiomycete, which may be responsible for one of the commonest rots in Manitoulin poplar (Fig. 1), was obtained from many specimens; in this study it has been designated as *Fungus X* and is discussed below.

In addition to the true tree-rot fungi the following Basidiomycetes which are commonly found in felled timber were obtained:

Merulius tremellosus (Schrad.) Fr.,
Phlebia strigosa-zonata Schw.,
Polyporus adustus (Willd) Fr.,
Polyporus versicolor (L.) Fr.,
Trechispora raduloides (Karst.) Reg.

A large number of Hyphomycetes were also isolated.

The rots peculiar to the living tree are discussed in this paper only in respect to their presence and activity in pulpwood in storage.

Logs for Storage Piles

In January, 1951, a survey was made of pulpwood being cut on Manitoulin Island with a view to becoming familiar with the types of rot present in the area. Bolts were then selected to supply enough samples of the various types to build both a field pile and a smaller pile to be stored in a pit below ground level; for the former, 76 logs were chosen, for the latter, 28. In January, 1952, a similar selection was made: 86 bolts for a field pile, and 18 for a pit. Logs for field storage were piled as described below; those for the pits were shipped to Ottawa.

It was thought that some idea of the loss in dry weight of the bolts during storage might be obtained by basing their original and final dry weights on that of disks cut at the time of piling and dismantling. At the outset, therefore, a disk about 1 in. thick was sawn for moisture content determination from the end of each bolt destined for field storage; if it was thought that the wood had been cut long enough for end-drying to have occurred, a thin disk was first sawn from the end and discarded. The remaining portion of the log, which was to be placed in the pile, was then weighed. The moisture content of the disk was found by the usual method: weighing, drying to constant weight, and calculating from the wet and dry weights. This moisture content was used as applicable to the log placed in the pile and the total dry weight was estimated. When the pile was dismantled each log was weighed; a 1 in. disk was sawn from halfway between the middle and one end of the log; the end section, about 1 ft. in length, was discarded, and the disk used for moisture content determination. The appropriate weighings were made, and the dry weight of the log estimated.

When the pit logs were received in Ottawa, a section 8 to 9 in. in length was sawn from one end of each log to provide wood from which cultures could be made; a 1 in. disk was then sawn off for moisture content determination. When the moisture content was calculated, the dry weight of the remainder of the log, the portion which was to be stored, was estimated as in the case of the field logs. At the conclusion of the test the final dry weight was also estimated from a disk sawn from halfway from the middle to one end.

Comparison of the original and final dry weights obtained showed immediately that the method was unsatisfactory; even for straight logs which were free from knots, the results were too irregular for acceptance. Most of the logs contained rot from the tree, which would doubtless account for considerable irregularity in moisture content throughout the bolt. Lack of uniformity in the bolts due to knots, wounds, decay, bark, and possibly other factors, made the results too variable for consideration.

Tree-rot Fungi in Storage

An attempt was made to identify the heartwood-rot fungi which were alive in the poplar selected for storage by culturing from the sections sawn from the ends of the pit logs. From the field piles cultures were made from disks from a few logs, but for the most part only the fungi which survived storage can be dealt with. Cultures were made from all the logs after the piles were dismantled.

Pit Storage

Storage in pits was decided upon with a view to providing a high moisture content such as might be found in the bottom of a mill block pile. The 28 bolts selected in 1951 were received in Ottawa in February. After the 8 to 9 in. sections and moisture content disks were removed, and the required weighings made, the remaining lengths were piled in the open and covered with a tarpaulin until spring. In April, a pit 8 ft. × 8 ft. × 5½ ft. deep was dug, and in it a rack for the bolts was placed. In this, the logs were piled so that none were in contact with the soil; sufficient space was allowed for examination of the bolts as required (Fig. 6). A thermohygrograph was placed in the pit in order to provide a record of temperature and humidity during storage. The pit was covered with boards, but not so closely as to check all ventilation; some boards were left loose for removal as occasion demanded.

A second pit was established in 1952. For this, 18 logs were obtained in January and handled in the same way as the 1951 logs; in April they were placed in a pit dug beside the first. Both piles were left until September, 1952, thus providing wood stored for two and one summer seasons; they were then dismantled and the condition of each log recorded in detail.

Although pit storage may be of less direct practical importance than storage in the field, it is here placed first because the cultures made from this material on arrival furnished information on the identity and viability of the fungi in the wood before storage. The results of the initial cultures from the two lots (1951 and 1952), 46 logs in all, are grouped together, since they supplied the viable fungi obtained from freshly felled poplar.

The end sections from the 46 bolts were ripped to expose fresh longitudinal surfaces; some 700 cultures were made in 1951 from the 28 logs received, and approximately 500 from the 18 obtained in 1952. When the piles were dismantled in September, 1952, representative sections were sawn off and ripped. Cultures to the number of 1150 were made from the logs from the larger pit, and 825 from those of the smaller. These cultures showed the reaction of the original fungi to storage and the penetration of true storage fungi which are discussed later in the paper.

Fomes igniarius var. *populinus* (Neu.) Campb. Rot caused by this fungus has been found prevalent in poplar in widely separated districts (3, 9, 15, 16); Manitoulin poplar is no exception; the rot was probably the most common type in the wood under study. It is a white rot, more or less punky according

to the stage of decay, and characterized by yellowish-green to black zone lines. Of the 46 logs, eight contained this rot, six in the larger and two in the smaller pile. The fungus was isolated, however, from three logs only, which suggests that it is dead in much of the rot seen in pulpwood piles. *F. igniarius* survived in one of the three bolts during two seasons in the pit, and even up to March, 1953, when a final examination and culture test were made after an additional winter in the storage shed; it was not obtained from the other two after the first culturing in March, 1951. Riley (15) reported that *F. igniarius* produced sporophores on felled poplar trees during a six-year period in which they were under observation; these trees, however, were left intact as felled. In the wood here considered the fungus does not appear to have caused appreciable change during storage.

Radulum casearium (Morgan) Lloyd. This fungus was first reported by Black (3) as the cause of a common rot of poplar in the Pic region of Ontario; it is also prevalent in the poplar pulpwood cut on Manitoulin Island. It produces a well-defined diffusion zone on gallic acid and shows a slight reaction on tannic acid; it is, therefore, classed with the white rots. The wood becomes yellowish in color, but often contains considerable red discoloration. Similar red wood is found associated with knots, wounds, and some other types of rot in poplar; the cause of the discoloration is not known, but it seems to develop in response to factors causing injury or irregularities of growth. With *R. casearium* rot the red is frequently marginal, but it may also occur in irregular pipes.

This rot was present in nine of the 46 logs, five in the large pit and four in the small. The fungus was not isolated from the rot in two of the five bolts of the 1951 pile; and from one, it was obtained only in the original cultures made in March, 1951; in the other two, it was alive after two seasons' storage. It was viable in all four of the 1952 logs at the conclusion of the test, that is after one summer season. Although *R. casearium* persisted in a majority of the logs during storage, the rot was not visibly altered; the fungus was either dormant or very slightly active.

Polyporus dryophilus var. *vulpinus* (Fr.) Overh. The 1951 logs did not contain decay caused by this fungus, but it was present in four of the 1952 bolts; it also reacts as a white rot. The wood is yellowish, punky to shreddy in advanced stages, pitted with numerous tiny holes, and sometimes mottled with fine dark lines. The isolates may at first show considerable variation in culture: some are smooth, brown, and stain the agar a dark brown; but all eventually produce soft balls of aerial growth, creamy to yellow-orange in color, with the agar usually bleaching and often penetrated by dark lines or plates; several cultures produced fertile but abnormal fructifications. The writer is indebted to Dr. M. K. Nobles, Canada Department of Agriculture, for identification of the culture. Of the bolts which contained the rot, three yielded the fungus in culture in February, 1952; it was still viable in two of these bolts in October after a summer's storage; but, owing to invasion of

the rot by other fungi, it was obtained from a smaller percentage of the cultures than in the spring. It does not appear to develop appreciably during storage.

Armillaria mellea (Vahl.) Quél. causes a butt rot in poplar which is fairly common on Manitoulin Island; it also is a white rot (5). It appeared in one of the pit logs only, a 1952 bolt. A small irregular pipe of advanced, shredded rot, traversed by fine dark strands, extended part way through the log. *A. mellea* was dead in the shredded wood, but was grown from one of the dark strands, thus permitting identification of the rot. The fungus did not survive storage.

Poria cocos (Schw.) Wolf. This fungus was first reported in Canada by Redmond and Robinson (14) as the cause of a butt rot of *Betula lutea* Michx. f. in the Maritime Provinces; it is quite common in Manitoulin poplar. The first sporophores reported in Canada were found on poplar pulpwood (7). The decay is a light brown cubical rot found in butt logs and also associated with wounds along the trunk; in advanced stages the fissures in the rot are filled with thick felts of mycelium and the wood is friable. The cultural characteristics have been described by Davidson *et al.* (6).

Of the 28 logs in the 1951 pile, *P. cocos* was found in one only when the pile was set up; it was the only fungus isolated from the section tested. In the autumn of 1952, it was still the only fungus obtained from that log and was found to have formed heavy mycelial felts over a considerable area of ends and bark; when the log was finally further dissected in March, 1953, the rot was found uniform through the sections. When cultures were made from all the pit logs in the autumn of 1952, it was seen that *P. cocos* had spread from the originally infected log into two others which had been in contact with it in the pile; both of these bore typical brown felts and yielded the fungus in culture. One contained a core of *Radulum casearium* rot in which that fungus had died out; in this log, *P. cocos* had penetrated the sapwood only, but subsequent observations have shown that it would have been capable in time of penetrating the entire log. The second bolt had contained a faintly gray heartwood, which had yielded Hyphomycetes only; in this, *P. cocos* had penetrated deeply.

One other log of this lot became infected with *P. cocos* but the infection occurred subsequent to the dismantling of the pile, and the direct channel by which it took place could not be traced. Two rots were present in the log at the outset and the causal agents were isolated: *Fomes igniarius* and *Pleurotus subareolatus*. After the pile was dismantled in September, 1952, and the bolts examined, the log was left in the storage shed until March, 1953, when it was further dissected in order to trace the two rots, but the cultures made yielded only *Fungus X* and *Hyphomycetes;* when the log was finally examined and dissected in October, 1953, it was heavily infected with *P. cocos;* the fungus had produced heavy surface felts, and was obtained in pure culture from the rot. *P. cocos* was rampant in the wood in the shed; it evidently found conditions during the hot summer of 1953 ideal for growth, and had attacked several logs which had originally contained other types of decay, including this 1951 pit log.

P. cocos rot with the fungus active was present in three of the 1952 pit logs when the pile was established. The fungus grew vigorously in these logs in the pit, and produced thick mycelial felts on end and bark surfaces (Fig. 8). Rot in two of the bolts was very advanced in October, 1952; ring shake and other fissures were present, and mycelial sheets were well developed. No evidence was obtained to indicate a spread of the fungus to other logs in the pit.

Fungus X was found associated with a characteristic heartwood rot (Fig. 1) in two of the 1951 logs. The viability of its mycelium in October, 1952, is shown at the margin on the rot on a cross section of a disk sawn from one of these bolts (Fig. 2). The disk, sawn from the bolt immediately after its removal from the pit, was kept in the laboratory for preliminary drying; the fungus grew out readily on the moist wood.

Pleurotus subareolatus Peck, which causes a white rot in poplar and other hardwoods, seems to be of minor importance in Manitoulin poplar. It occurred with *Fomes igniarius* in one of the 1951 pit logs, and it survived two seasons' storage without spreading or causing appreciable damage.

The remaining pit logs, 14 of 1951 and 4 of 1952, yielded no tree-rot fungi; 10 were practically free of rot at the outset, and from the remainder Hyphomycetes only were isolated.

Field Storage

Logs for the storage piles were selected with a view to determining the reaction of the several tree-rot fungi to field storage conditions, and to following the development of storage-rot fungi in the piles. The first pile was established in January, 1951, and the second in January, 1952; both were dismantled in October, 1952, thus providing wood stored for two and one summer seasons respectively. Bolts were selected representing all types of rot available in the cutting area at the time of piling.

Results for the two piles are not entirely comparable because of differences in piling-site conditions. At the outset, owing to reports of extensive decay during prolonged storage in mill block piles, attention was directed to conditions as provocative of decay as possible; the site chosen was, therefore, a shady spot under a hardwood canopy. During the following year the enforced storage of large quantities of wood for lengthy periods at loading points on open beaches was emphasized. This led to the placing of the 1952 pile at the edge of an open beach where it was exposed to the prevailing winds; it was put up in the neighborhood of commercial piles containing some 2000 cords which were being held over.

The 1951 and 1952 piles contained 76 and 86 bolts respectively, each bolt about 50 in. long; they were representative of the rots prevalent in the region. They were piled unbarked in corded ranks, the different types of rot being distributed through the piles. When examined in July, 1951, the pile was well exposed to the sun owing to the defoliation of the trees by the tent caterpillar; later in the season, however, new foliage developed and provided the desired shelter.

As stated above, at the end of the test a disk for moisture content determination was sawn from each bolt from halfway between the middle and one end. The smaller section, about one foot in length, was discarded; the longer portion, about three-quarters of the log, was shipped to Ottawa for study. The two lots on arrival were piled separately in the open and covered with tarpaulins, but a few weeks later they were removed to a well-ventilated storage shed so that they would be more readily accessible for study during the winter.

The work was interrupted, and it was not until March, 1953, that an analysis of the logs was commenced. They were handled in the order in which they became accessible, and each was sawn to expose the rot present. First a disk was sawn from the end which had been exposed in the field; all but two of the 1951 disks bore sporophores of storage-rot fungi, and about 50% of those from the 1952 pile; had the other ends not been discarded it is probable that the number of bolts recorded as bearing fruit bodies would have been increased. The remaining portion of each log was then sawn into three approximately equal lengths, and the four pieces numbered one to four, beginning with the disk. From a majority of the logs sections two and three were then ripped to expose the wood in longitudinal section; according to the appearance of the interior one or two lengths were ripped out. From the 1951 bolts some 2800 cultures were set up on malt agar slants during March and April; some 3300 were made from the logs of the second pile during April and May. The sections of each bolt were returned to the shed after culturing, and were piled on top of one another in upright position in order 4, 1, 2, 3; owing to further interruption of the work the sections remained undisturbed until the autumn of 1953, and some were not reached until January, 1954. In the meantime, however, the cultures had been examined and records made.

Fomes igniarius var. *populinus* rot was present in four of the 1951, and in 14 of the 1952 bolts. The fungus was isolated from two of the 1951 logs, and during the summer of 1953 the mycelium grew out sparingly on cut surfaces of a third; in the fourth it was quite inert. Of the 14 bolts of 1952, *F. igniarius* was obtained in culture from 11; in the autumn small patches of the mycelium of the fungus were noted on longitudinal faces of three of these. *F. igniarius* had not caused appreciable damage during storage, and showed gradually decreasing vitality.

Radulum casearium was the most prevalent type of rot in both field piles; it was recognized in 20 of the 76 bolts of 1951, and in 27 of the 86 selected in 1952. In the spring of 1953 the fungus was isolated from 11 of the former, and from 22 of the latter. This shows a much higher percentage of the 1952 bolts with viable mycelium, which suggests a loss of vitality with lengthened storage. In the autumn of 1953 after summer storage in the shed, surface growth of *R. casearium* mycelium was found on longitudinal faces of 3 of the 11 bolts of 1951 and of 10 of the 22 from the 1952 pile; this indicates reduction of the activity of the fungus with the drying of the wood. Judging from the appearance of the disks taken from the bolts at the time of piling and of the

wood when finally examined there is no evidence of appreciable deterioration due to this fungus during storage.

Polyporus dryophilus var. *vulpinus* rot was present in only one of the 1951 bolts; the decay was advanced, but no surface growth of mycelium developed, nor was the fungus isolated in culture; it is possible that the fungus was dead when the bolt was placed in the pile. Of the 86 logs in the 1952 pile, 13 contained advanced *P. dryophilus* var. *vulpinus* rot; the fungus had been isolated from disks taken from three of these when the pile was established, the only bolts tested at the time. It was obtained from all 13 in the spring of 1953, and in the autumn its mycelium was noted in seven on the longitudinal faces exposed in sawing the sections for cultural analysis. There was no external mycelial growth which would enable the fungus to spread in the pile. This fungus also seemed to be of little or no importance in causing storage rot.

Poria cocos rot was present in four of the 1951 bolts and the fungus grew in culture from inocula taken from points throughout the affected areas; in three there was extensive central rot, while in the fourth, the decay was in the sapwood. In the autumn of 1953, it was found that *P. cocos* had grown vigorously in the four bolts during the summer and had invaded two other logs, one of which had originally contained *Radulum casearium* rot and the other the gray–brown butt rot mentioned above as producing a culture characterized by the production of fine brown strands. *P. cocos* had produced thick, strong felts on the bottom of No. 4 sections in contact with the floor of the shed, and strap-like felts up along the sides. The sections of one log were firmly held together by thick growths of the fungus (Fig. 7) between the pieces; thinner felts were noted on surfaces, both transverse and longitudinal, of other bolts; the vigor of mycelial growth varied with the moisture content of the wood, being less luxuriant on drier pieces. The fungus had spread during the summer to the bolt containing *R. casearium* rot, had penetrated to the rot core, and, to the extent of its penetration, had obliterated the original rot, bleaching it to the characteristic light yellow-brown *P. cocos* rot; the fungus had similarly penetrated the gray–brown butt rot. *P. cocos* also produced fruit bodies around the base of section 4 of the bolt containing *R. casearium* rot.

Extensive *P. cocos* rot was present in eight of the 1952 logs in the spring of 1953, and was readily isolated from all. By the autumn of 1953, after summer storage in the shed, the fungus was rampant in these bolts; thick leathery felts had formed on the bottoms of the sections in contact with the floor, and sections and parts of sections were firmly locked together by sheets of mycelium such as are shown in Fig. 7; the lower sections were completely rotted, the fungus being checked above by the drying of the wood. *P. cocos* had fruited around the base of one log. In addition, the fungus had spread to seven bolts in which it had not been present in the spring. One of these had previously yielded *Fomes igniarius*, but the original rot core was invaded by *P. cocos*, and inocula from the area penetrated produced the latter fungus in October; similar isolations were obtained from an invaded *Radulum casearium*

core, and one of the gray–brown butt rot. *P. cocos* had also spread extensively in four bolts from which no primary wood-rot fungus had been obtained in the spring; it had grown vigorously forming external felts and producing advanced decay.

The results show that the tree-rot fungus, *P. cocos*, may cause extensive decay in storage piles. It produces a brown rot, which indicates a destructive action on cellulose; it would be expected, therefore, to reduce greatly the yield of both groundwood and chemical pulp.

Corticium polygonium Pers. is fairly common in Manitoulin poplar, but whether or not it is the causal agent of a primary rot has not been established. It was isolated from 11 samples mentioned above as sent in from freshly felled trees; these samples came from heights ranging from 6 to 16 ft. in the trees. Small fruit bodies found on two of the samples were identified by Dr. R. Macrae, Canada Department of Agriculture; the isolate from adjacent wood was then recognized by comparison with cultures from the sporophores. The fungus is associated with trunk rot and enters through branch stubs or wounds. *C. polygonium* was isolated from six of the 1951 bolts, but was not obtained from any in the 1952 pile. From the lack of uniformity in the appearance of the wood from which it was obtained it seems doubtful that it causes a primary rot. In one bolt it was associated with *Radulum casearium*, in another with *Fungus X;* it was obtained from a dark core which it had apparently entered through a branch stub; in two, it came from yellowed sapwood, the infected wood in one being below a wound; finally it was isolated from a mottled red–yellow core, which may have originally contained *R. casearium*. The fungus does not appear to cause damage during storage.

Fungus X was isolated from seven of the 1951 bolts, all of which contained cores of the characteristic rot pattern; it was obtained in culture from throughout the affected areas. Two of the 1952 bolts contained similar cores, and one other an irregular rot not concentrated in a solid core. The same fungus, as judged by cultural characteristics, was isolated from the sapwood or outer heartwood of 41 additional 1951 logs, and from 23 of the 1952 pile; it was also obtained from one bolt containing viable *Fomes igniarius*. Since it appeared in the sapwood of a much higher percentage of the 1951 than of the 1952 bolts, it would appear that it may attack wood in storage as well as in the living tree. There was no evidence, however, of its causing serious damage in the piles.

The remaining tree-rot fungi isolated were of minor importance in the piles; they did not spread, but appeared to remain confined to the logs infected at the outset. *Pholiota spectabilis* Fr. (Quél.), which causes a brown butt rot, was found in one bolt in each pile; it was isolated from a 1951 log in the spring of 1953, and the following October it produced two fruit bodies on a transverse surface of the rot core of a 1952 bolt. *Pholiota adiposa*, a white rot fungus, was present with *P. spectabilis* in the 1952 bolt, and was isolated from one other log in the same pile. Both of these fungi infect the living tree and are able to survive and fruit on felled timber; they did not spread in the piles

under study, and it is unlikely that they cause appreciable loss during storage. *Pleurotus subareolatus* Peck, which also causes a white rot, was not isolated from the 1951 logs, but it was found fruiting on one of them when the pile was dismantled. It was isolated from a disk cut from one of the 1952 bolts when the pile was being set up, and from the same log in the spring of 1953; the 1952 culture produced fertile fruit bodies which permitted specific identification. The fungus did not spread in storage. The fungus which causes the *gray-brown butt rot* was isolated from one 1951 bolt, and from two of the 1952 pile; the rot was also recognized in a third 1952 log. It was still active in one of the 1952 logs in the autumn of 1953 and had probably continued rotting the log during storage; but it had not spread in the pile.

A considerable number of bolts provided no specific information as to the reaction of the tree-rot fungi to storage conditions. Of the 76 bolts in the 1951 pile, 21 yielded no tree-rot fungi in culture although they contained definite but unidentified heartwood rots; eight of the 86 bolts of 1952 reacted similarly. The tree-rot fungi were either dead at the outset or died out during storage. Since the fungi in 27% of the logs of the two-year pile were inactive as opposed to 9% of those stored for one season, it is reasonable to assume that the tree-rot fungi gradually lost vitality during storage. The penetration of storage-rot fungi is discussed below; these organisms would doubtless hasten for the gradual dying out of the species originally present.

Some logs in both piles, 14 in the 1951 lot and 7 in the 1952, contained no heartwood rot; these furnish information regarding storage fungi only.

Block Pile Storage

The poplar block pile examined was of the ordinary type in which logs are dropped from an overhead conveyor to form a more or less conical pile. The bolts are received unbarked, but in the course of handling and storage much bark becomes detached and clogs the pile. The wood is sprayed with water as a protection against fire, and this maintains a moisture content which seems favorable for fungal growth. Attention was concentrated on a portion of the pile which had been in storage for three years. Sporophores of fungi covered the logs; it seemed a veritable fungus garden. Owing to the structure of the pile only surface logs toward the base could be examined; samples of 16 bolts were obtained for study. The wood was examined for heartwood rots, which would have originated in the living tree; 11 of the 16 logs contained such decay. Four of these contained rot of the *Radulum casearium* type, and the fungus was isolated from one of them; *Fomes igniarius* rot was present in one sample and yielded the fungus in culture. The rot of characteristic pattern with which *Fungus X* has been associated was found in two logs and the fungus was readily obtained in culture. *Pholiota adiposa* was fruiting on one log and was cultured from the wood, which was thoroughly rotted. *Pholiota destruens* (Brond.) Quél., which has been reported (17) as the cause of one of the most prevalent heartwood rots of black cottonwood in British Columbia, was fruiting on one log, and was undoubtedly responsible for the

original heartwood decay; this is the only record of this fungus in the storage piles, but it was obtained, as noted above, from the samples received in 1951. It may be more prevalent than results have indicated, since it grows very slowly on malt agar, and may have failed to spread from some inocula in which it was alive. Two heartwood rots were not identified either by pattern or isolation of the causal agents. The remaining five bolts did not contain heartwood rots; they furnished information only on the presence of fungi which entered during storage.

Storage-rot Fungi

During the warm summer weather fruit bodies of storage-rot fungi began to appear on logs in all the piles, which indicated that the wood was being rapidly attacked by a new set of fungi.

Pit Storage

Temperature and humidity were recorded in the larger pit from May 10 to the end of October, 1951, and from May 1 until Sept. 15, 1952. In May, 1951, the temperature rose from a low of 34° F. to a high of 58°, remaining for the most part in the low fifties; the high in June was 62°, in July 66°, in August 68°, after which it dropped gradually with fluctuations to a low of 47° at the end of October. The range in 1952 was slightly, though not significantly higher, the lowest temperature recorded being 44° F. and the highest 74° F. The relative humidity was high during both seasons, rarely dropping below 90% and usually being in the neighborhood of 100%.

In the smaller pit the conditions of temperature and humidity were very similar to those recorded in the larger during the summer of 1952. The record was kept from June 2 to Sept. 20; during that period temperature range in the larger pit was 56°-74° F., and in the smaller 56°-70°. The relative humidity was similar also, for the most part approaching saturation.

By the middle of August, 1951, *Stereum purpureum* Pers. was beginning to fruit on the ends of several logs, and a small fruit body of *Schizophyllum commune* Fr. was noted on one bolt. The latter fungus did not develop further in the pile, but by the end of August, *S. purpureum* sporophores were well developed; they continued to increase in number and maturity until the end of the season, at which time they were present on a majority of the logs. *Collybia velutipes* (Curt.) Fr. fruited on three logs in the autumn of 1951, and on one of these in the early spring of 1952. This fungus probably came in from the living tree; it causes a white rot of dead sapwood in living trees as well as in fallen logs, but does not spread extensively; it fruits readily in late autumn or early spring when temperatures are low.

When the piles were dismantled in September, 1952, it was found that *Stereum purpureum* (Fig. 9) had fruited on every log in the 1952 pit, and on all but two of the 1951 logs. Of these two, one bore heavy felts of *Poria cocos*, and was well rotted by that fungus, which seems sufficiently hardy to maintain itself in opposition to other species. No other fungi had fruited on the 1952 logs, but *P. cocos* felts were found on three (Fig. 8).

During the second storage season *Polyporus adustus* (Willd) Fr. fruited on 12 logs, *P. hirsutus* (Wulf.) Fr. on 13, and *P. versicolor* (L.) Fr. on 4; these fungi cause white rots, attacking first the sapwood, but capable of rotting the entire log. Fig. 10 illustrates the luxuriance with which fruiting had occurred by the end of the second season of pit storage; this log bears sporophores of *S. purpureum*, *P. hirsutus*, and *P. versicolor*. Although the three species of Polyporus had not fruited on the 1952 logs, their appearance in cultures showed that even in the first year they were becoming established in the wood; *P. adustus* and *P. hirsutus* were both present with *S. purpureum* in two logs, the former was found in three other bolts, and the latter in one. *S. purpureum* was isolated from all but one of the one-season logs, that one being completely penetrated by *Poria cocos*, and it was cultured from 18 of the 28 bolts of 1951. It was the first fungus to penetrate the logs, but was gradually replaced by the three species of *Polyporus* and other fungi. *P. adustus*, *P. hirsutus*, and *P. versicolor* were not obtained from every log on which they fruited, but from the combined evidence of fruiting and cultures it was established that *P. adustus* had attacked 23, *P. hirsutus* 22, and *P. versicolor* 7 of the 28 logs; in six, all three were alive in addition to *S. purpureum*.

Three of the 28 bolts were attacked by *Poria cocos* and were relatively free of other fungi; one in which it had been present at the outset yielded no other fungus in culture. Two logs which were in contact in the pile with the *P. cocos* log had been infected, one extensively. This last bore fruit bodies of *S. purpureum*, *P. adustus*, and *P. hirsutus*, but of the three, *P. adustus* only grew in culture; the other log still contained viable mycelium of each of the three with *P. cocos* encroaching upon them. A fourth log became infected with *P. cocos* in the shed; *P. adustus* was alive in it when the pile was dismantled, but the following year, October, 1953, *P. cocos* only was isolated.

Field Storage

When the pile established in January, 1951, was examined the following July, sporophores of *Stereum purpureum* were beginning to develop on the ends of some of the logs; by September the fungus had fruited on practically all of them. Although the pile established the next year was on a more exposed site, *S. purpureum* fruitification followed a similar pattern; by the autumn the fungus was fruiting on more than half of the logs. During the second season it continued to produce fresh sporophores on the 1951 bolts. Combining culture and fruit body records it was found that *S. purpureum* developed in all but one of the 1951 bolts and in all but four of the 1952. *Poria cocos* tended to check the entrance of other fungi; three 1951 logs containing *P. cocos*, although they bore fruit bodies of *S. purpureum*, did not yield that fungus in culture, and two 1952 logs contained *P. cocos* only.

With the exception of one small fruit body of *Phlebia strigosa-zonata* on a 1952 log, no sporophores other than those of *S. purpureum* were found in either pile during one season's storage; but when the piles were dismantled in October, 1952, sporophores of several other species were observed on the 1951

logs. *Polyporus hirsutus* had fruited on 16 bolts, *Phlebia strigosa-zonata* (Schw.) Lloyd on three, and *Pleurotus subareolatus* and *Polyporus pargamenus* Fr. each on one. One log bore sporophores of four fungi: *S. purpureum*, *Phlebia strigosa-zonata*, *Polyporus hirsutus*, and *P. pargamenus;* on the other two logs on which *Phlebia* developed, fruit bodies of *S. purpureum* and *P. hirsutus* were also found.

The pattern of penetration in the field was similar to that in the pits. By cultural analysis it was found that the storage-rot fungi had become established in the 86 logs of 1952 as follows: *Polyporus adustus* in 25, *P. hirsutus* in 21, *P. versicolor* in one, *Phlebia strigosa-zonata* in two, and *Corticium laeve* Pers. in 15. Another Basidiomycete, *Trechispora brinkmannii* (Bres.) Rog. and Jacks. was isolated from two, but it is doubtful that this fungus attacks the wood; in soil–block culture it caused no loss in weight in wood blocks during four and a half months' incubation. Of the 76 bolts in the 1951 pile, *Polyporus adustus* was present in 20, *P. hirsutus* in 27, *P. versicolor* in eight, *P. pargamenus* in four, *Phlebia strigosa-zonata* in four, *Corticium laeve* in 12, and *Trechispora brinkmannii* in four. Storage decay had increased in extent and severity; Fig. 5 shows advanced *P. hirsutus* rot from one of the logs. Three logs which contained *Poria cocos* rot at the outset did not yield any of the storage-rot fungi in culture.

Block Pile Storage

As stated above the block pile examined in October, 1952, presented a very luxuriant growth of fruit bodies. *Polyporus hirsutus* was the predominant form; *Stereum purpureum*, *P. adustus*, and *Schizophyllum commune* were common; a more thorough search would doubtless have provided specimens of other species. The time available for the survey was short, and samples from only 16 logs were obtained. From these, *S. purpureum*, *P. adustus*, *P. hirsutus*, *Collybia velutipes*, and *Trechispora brinkmannii* were isolated. The results indicated a fungal flora similar to that reported above. The tree-rots in these logs have been discussed above; no evidence was obtained to indicate that they had spread beyond the logs in which they were originally present, but the condition of two logs, one rotted by *Pholiota adiposa* and the second by an unidentified fungus, suggested that these two species may have continued to deteriorate these bolts in the pile. Apart from the heartwood rots the wood was unexpectedly clean; the samples were, however, too few to provide a general picture of decay in the pile as a whole.

Fungus X

The status of *Fungus X* in relation to heartwood decay requires study. It is frequently isolated in uniform series from entire cores of a rot of very characteristic pattern (Fig. 1), but it also occurs in sapwood and in association with other types of decay; it may be a primary heartwood rot fungus with ability to spread in stored wood, or it may be secondary in a succession of organisms as has been suggested by Good (8) for *Corticium vellereum* Ell. and Cr. in maple.

On malt agar the fungus produces a white to cream or slightly yellow mat, the aerial growth at first appearing rather soft and often showing rounded, radiating ridges; although superficially soft, the mat is usually tenacious and difficult to cut with an inoculating needle; the aerial growth soon becomes appressed to the agar, sometimes leaving a patchy surface. One of the isolates which sporulated was submitted to Dr. J. W. Groves, Canada Department of Agriculture, and identified by him as probably a species of *Libertella*. Conidia in this and in other isolates were hyaline, long, filamentous, and curved, as in that genus (4). Most of the isolates did not sporulate, and most of those which did soon became sterile on transferring.

The ability of *Fungus X* to cause decay in poplar heartwood was tested by the soil–block method. Three blocks 1 in. × 1½ in. × ⅝ in. were sawn in sequence from carefully machined sticks of poplar heartwood. The moisture content of the median block was determined and considered applicable to the sister blocks. The latter were buried in damp soil (300 gm. soil, 75 cc. distilled water) in culture jars fitted with screw tops. The assembly was sterilized at 15 lb. pressure for one-half hour; four jars, each with two blocks, were used. Inoculum was taken from two isolates of *Fungus X*, each being used for a pair of jars. The cultures were incubated at 78°-80° F. for four and a half months; loss in dry weight of the blocks was then calculated. Both isolates were effective in causing decay; loss in three jars was about uniform and showed a reduction in dry weight of 32%. In the fourth jar the fungus did not grow as well, the loss being only 14%; this reduced the over-all average loss to 27.5%. The test demonstrated the ability of *Fungus X* to attack sound poplar.

The interaction between *Fungus X* and some of the common tree-rot and storage-rot fungi was tested on malt agar plates. Two strains of *Fungus X* were used with each of the following fungi: group 1—*Fomes igniarius* var. *populinus*, *Radulum casearium*, *Polyporus dryophilus* var. *vulpinus*, and *Corticium polygonium;* group 2—*Stereum purpureum*, *Polyporus adustus*, and *Phlebia strigosa-zonata*. Fresh cultures of each fungus were grown on plates containing equal depths of malt agar. When the mycelium was developing actively, inocula were punched out with a metal tube from toward the periphery of the mats and transferred to fresh plates as follows: an inoculum of *Fungus X* was placed midway between the center and circumference of each plate; in the same position on the opposite side of the plate was placed a similar inoculum of the fungus to be tested. Cultures were set up in triplicate with each strain of *Fungus X*. The fungi were allowed to develop for four to seven weeks according to the vigor of their growth; at the end of seven weeks the plates had dried to a point where development had ceased. Transfers were made from each plate to malt agar slants in order to determine the viability of the fungi; inocula from both surface and submersed mycelium were tested. Following are the results for the several fungi:

Fomes igniarius grew slowly and was finally covered by *Fungus X* in all the plates; the latter fungus alone survived at the end of the test.

Radulum casearium and *Fungus X* grew with approximately equal rapidity. In one week each had covered about half of the plate and had formed a marginal thickening at the edge of a clear, narrow dividing zone. After this, neither fungus advanced appreciably as far as could be judged by visual examination (Fig. 4). Both fungi were recovered from their respective sides in all but one of the plates; in that one, *R. casearium* was obtained from near the *Fungus X* inoculum.

Polyporus dryophilus var. *vulpinus* and *Fungus X* grew to contact in one week, the latter growing more rapidly and occupying about two-thirds of the plate. Superficially the cultures did not change with age. At the end of a month *P. dryophilus* var. *vulpinus* grew from inocula from the submersed mycelium, but plates tested at the end of seven weeks yielded *Fungus X* in all the tubes.

Corticium polygonium covered about one-quarter of the plate in one week, while *Fungus X* occupied the remainder; the line of contact was sharp. Later, *Fungus X* tended to cover *C. polygonium*, but at the final test, both fungi were alive.

Stereum purpureum grew more rapidly than *Fungus X*, covering about two-thirds of each plate in a week. Later it showed a slight tendency to overgrow *Fungus X*, but both fungi were isolated from their respective areas at the end of the test.

Polyporus adustus also grew more rapidly than *Fungus X*, covering two-thirds or more of each plate in a week. *P. adustus* completely covered the mats of one strain of *Fungus X* in a month, and continued to advance to almost cover the mats of the second before it was arrested by lowering of the moisture content. At seven weeks *Fungus X* was dead to the extent to which it had been covered, but was still viable in the exposed area.

Phlebia strigosa-zonata, in association with one strain of *Fungus X*, covered about one-half of each plate in the first week, with the other about two-fifths. At first there was a narrow clear zone between the two, but *P. strigosa-zonata* soon bridged the gap. In a month it had sent out fanlike growths to cover *Fungus X* in all the plates (Fig. 3). *Fungus X* was killed; *P. strigosa-zonata* was isolated pure from both submersed and surface mycelium on both sides of the plates.

Fungus X reacted unfavorably on two heartwood-rotting fungi, killing *F. igniarius* var. *populinus* and *P. dryophilus* var. *vulpinus*, but failed to check the third, *R. casearium*. With *C. polygonium*, whose status as a primary rot fungus is uncertain, it was slightly the more aggressive. The storage-rot fungi, on the other hand, tended to check *Fungus X; Phlebia strigosa-zonata* killed it in all the plates, *P. adustus* overgrew and killed it to a large extent, and *S. purpureum*, by growing rapidly, prevented its spread. The results suggest that *Fungus X* would tend to react unfavorably on the heartwood-rotting fungi, but would itself be checked by those entering during storage.

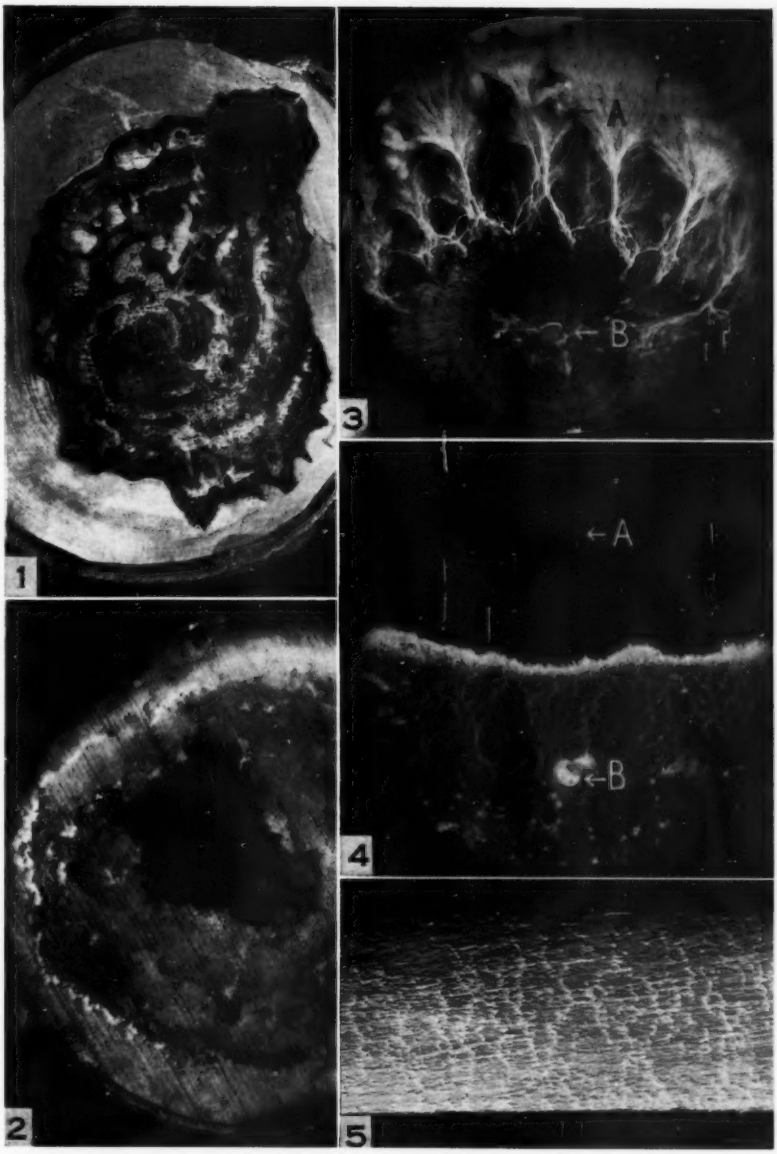

FIG. 1. Cross section of rot associated with *Fungus X*.
FIG. 2. Cross section of rot associated with *Fungus X* showing active mycelium at margin of rot.
FIG. 3. *A*, *Fungus X* overgrown by *B*, *Phlebia strigosa-zonata*, on a malt agar plate.
FIG. 4. *A*, *Fungus X*, and *B*, *Radulum casearium*, on a malt agar plate.
FIG. 5. *Polyporus hirsutus* rot in a 1951 field log.

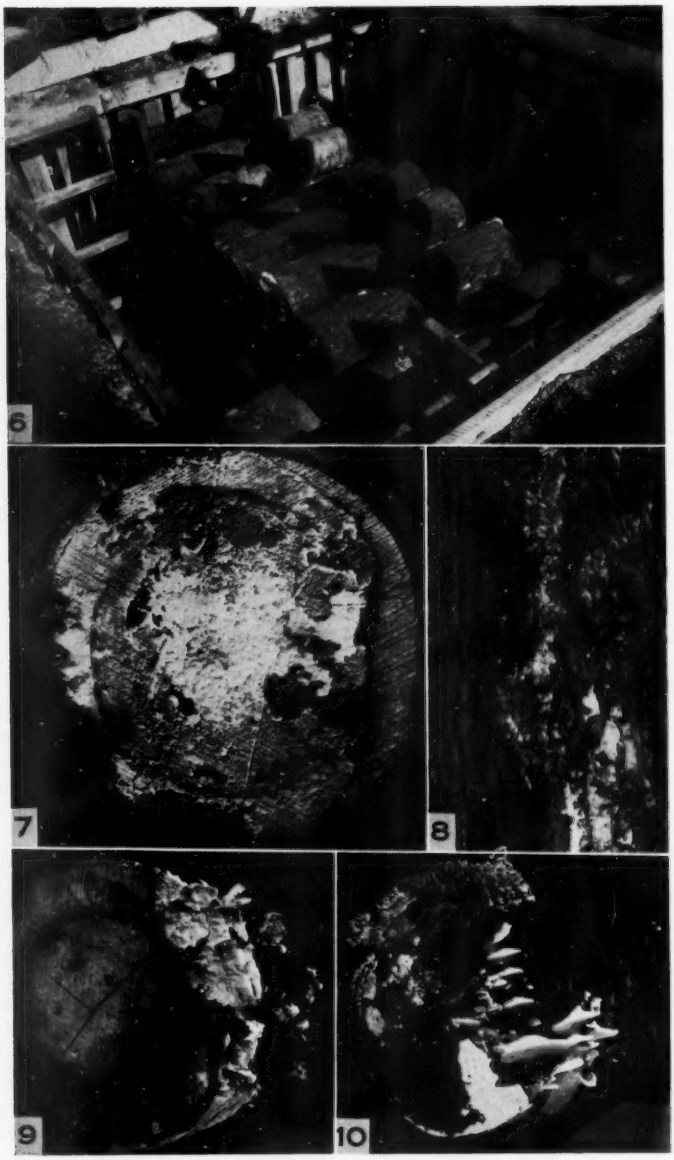

FIG. 6. 1951 pit pile before dismantling in September, 1952.
FIG. 7. *Poria cocos, mycelial* felt developed between sections of a pulpwood bolt.
FIG. 8. *Poria cocos*, mycelial felt on surface of a pit log.
FIG. 9. *Stereum purpureum*, sporophore on a 1952 pit log.
FIG. 10. *Stereum purpureum, Polyporus hirsutus*, and *P. versicolor* fruiting on a 1951 pit log.

Other Fungi

In addition to the wood-rotting fungi a number of *Hyphomycetes* and some *Ascomycetes* were obtained in the cultures, but no attempt was made to identify them. Some were constantly associated with a particular rot fungus, one such was found frequently in *Polyporus dryophilus* var. *vulpinus* rot. A few caused stains in heartwood rots, one producing a bright green, another a brilliant greenish-yellow discoloration. Blue stain developed to a certain extent in most of the logs during early storage; it was often obliterated in time by the development of rot. Apart from blue stain, discoloration developed for the most part in badly rotted wood, which would in any case have had little pulping value.

Discussion

The most prevalent of the tree-rot fungi encountered in this study, and the most important from the standpoint of the extent of decay in the trees attacked, were *Fomes igniarius* var. *populinus*, *Radulum casearium*, *Polyporus dryophilus* var. *vulpinus*, *Fungus X*, and *Poria cocos*. The first three cause white trunk rots. Even in the early stages of these rots the color of the wood is altered, and it is to be expected that a corresponding change might be found in groundwood pulp produced from them. Since in the early stages of white rots cellulose is not seriously attacked, their utilization for chemical pulp might be the more economical; both in chipping and grinding, however, losses would doubtless occur owing to weakening of the fiber. These fungi are not appreciably active in storage, so that the appearance of the respective rots as the bolts are cut should indicate their final condition as far as the original fungi are concerned. The rots are quite readily recognized and, if test runs were made to determine their pulping value, it should be possible to establish definite standards regarding their utilization. *Poria cocos*, on the other hand, is of the destructive type. It not only causes a brown rot thus destroying the cellulose, but it may grow vigorously during storage, being able to spread from log to log in the pile. It should be culled at the felling site.

Other tree-rot fungi isolated, *Pholiota adiposa*, *P. destruens*, *Pleurotus subareolatus*, and *Stereum murrayi*, were of sporadic occurrence and exhibited limited activity in storage; *Corticium polygonium*, whose status as a primary rot fungus is uncertain, reacted similarly. All these would appear to be of minor importance in their effect on pulp production. *Armillaria mellea*, *Pholiota spectabilis*, and the unidentified gray-brown butt rot, being confined to a few feet at the base of the bole, would also be accountable for only a small percentage of losses caused by decay.

The storage-rot fungi were limited in number and confined to white rot species in the wood studied. In all the piles *Stereum purpureum* entered first, but was beginning to be replaced by other species even during the first season. This differed from the condition found by Björkman (2) who was able to obtain aspen wood for pulping tests which had been rot-damaged in the open

for three years by *S. purpureum*. He found that the yield of sulphite pulp from the infected wood was less than 5% lower than that from sound wood as calculated on a volume basis, and the quality was slightly higher. That the fungus may, however, cause serious decay was shown by the results which he obtained on poplar blocks in pure culture; in two series of culture tests, each run for four months, *S. purpureum* caused a loss in dry weight of 36% and 42%.

The three species of *Polyporus*, *P. adustus*, *P. hirsutus*, and *P. versicolor*, which followed *S. purpureum*, are closely related to *P. zonatus*, which was common in Björkman's logs. *P. zonatus* caused a much more serious loss in yield than *S. purpureum;* sulphite pulping tests on comparable sticks showed a decrease in yield of 15.5% for *P. zonatus* as compared with 3.7% for *S. purpureum* considered on a volume basis, and 6.6% compared with 0.8% based on weight. It is probable that the three species of *Polyporus* found in the Ontario piles have a reaction similar to that of *P. zonatus*. In Fig. 5 is shown a sample of *P. hirsutus* rot from a bolt held for two summer seasons in ordinary field storage; from the appearance of the wood it is obvious that loss of substance would occur if such material were subjected to grinding or chipping.

Corticium laeve and *Phlebia strigosa-zonata*, which also effect decay in storage, caused some discoloration of the wood; the former produced a pinkish-orange color and the latter purplish streaks or mottling.

The investigation indicated that decay losses which accrue in storage are due for the most part to fungi which enter after the trees are felled; with the exception of *Poria cocos* the tree-rot fungi did not spread in the piles, and if they continued to grow damage was confined to the bolts in which they were originally present.

Comments

The economic losses due to decay in stored pulpwood have been under consideration for more than 50 years and the conditions of storage which favor decay are well known. In 1925 Kress *et al.* (10) discussed the question in a lengthy publication and summarized the information then available; they recommended the application of known principles of sanitation and rotation. Difficulties in transporation, high cost of labor, etc. have, however, prevented the handling of pulpwood in the manner recognized as desirable if full value is to be obtained from the wood cut. Björkman (1) has added to our information about many species of fungi found attacking stored pulpwood and has provided a valuable bibliography of more recent literature; investigators in the United States (11, 12, 13) have reported upon special studies made on localized pulpwood supplies. No method applicable to estimating losses due to decay in pulpwood as a whole has, however, been suggested, and it seems unlikely that such a method could be developed, since the number of variable factors entering into deterioration of wood in storage is so great, e.g. quality of wood from different age and site groups, types of decay, time of felling,

method of transport and storage, and climatic variation. If it were possible to estimate losses due to decay, it might be found economically desirable to spend more on protective measures; but after many years and an appreciable amount of work by many investigators on problems related to the subject, the best recommendation which can be made is rapid utilization of the wood after the trees have been felled.

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INVESTIGATIONS ON THE GENERA *BEAUVERIA* VUILL. AND *TRITIRACHIUM* LIMBER[1]

By D. M. MacLeod[2]

Abstract

A thorough review of the literature shows that 32 alleged species have been involved in the genera *Beauveria* and *Tritirachium*. Fourteen of these are reduced to synonymy with *B. bassiana* and *B. tenella;* ten belong to the form genus *Tritirachium;* one, "*Tritirachium epigaeum*", is regarded as uncertain as to genus; and seven are excluded from both. *Beauveria stephanoderis, B. laxa, B. globulifera, B. effusa, B. vexans, B. doryphorae, B. delacroixii,* and *B. acridiorum* are strains of *B. bassiana;* while *B. densa, B. melolonthae, B. brongniartii,* and *B. shiotae* are strains of *B. tenella.* The species *T. dependens, T. album, T. spicatum, T. oryzae, T. heimii, T. brumpti, T. musae, T. purpureum, T. cinnamomeum,* and *T. roseum* are characteristic of the form genus *Tritirachium. Beauveria peteloti, B. rileyi, B. paranense, B. coccorum, B. coccospora, T. rubrum,* and *T. viannai* are characteristic of neither genus. The above conclusions are based on a study of the cultural and morphological characteristics of numerous *Beauveria* isolates from 70 insect and four rodent species. Most characteristics adopted by earlier investigators to differentiate between *Beauveria* species are not valid as criteria upon which to establish species. Moreover, these characteristics are unstable and can be changed simply through monospore culturing or by transferring cultures from one type of medium to another. From a review of the literature, it is evident that members of the genus *Beauveria* are primarily parasitic on insects, whereas members of the genus *Tritirachium* are primarily saprophytes.

Introduction

Investigations on the morphology and taxonomy of species of the genus *Beauveria* were undertaken as part of a study of the problem of utilizing disease-producing organisms in the biological control of insect pests. The structural make-up and general polymorphism associated with the various parts of the fungus and its cultural characteristics in natural and artificial media have been investigated with a view to discriminating between intra- and inter-specific variability. In describing members of genera, it is obviously essential that species be distinguished from one another with certainty, especially since each may be associated with a specific host. Moreover, a knowledge of the morphology and classification of the various species may be expected to be of considerable importance in future investigations concerning various phases of the general host–parasitic relationship.

The discussion of *Tritirachium* species in this paper is not based upon a variability study of the different species but, rather, upon an examination of some *Tritirachium* cultures, and more particularly on a review of their descriptions and illustrations as published in the literature.

The genus *Beauveria* was established in 1912 by Vuillemin (145) in honor of Beauverie who, in 1911 (9), had pointed out that characteristics of this

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Contribution No. 164, Forest Biology Division, Science Service, Department of Agriculture, Ottawa, Canada.

[2] *Laboratory of Insect Pathology, Sault Ste. Marie, Ontario.*

group warranted its recognition as a distinct genus. Previously, the component species had been assigned to one of the genera *Botrytis* (5, 9), *Sporotrichum* (102, 128), or *Isaria* (48, 62).

The genus first came into prominence when one of its representatives, *Beauveria* (= *Botrytis*) *bassiana* (Bals.) Vuill., was identified (5) as the agent responsible for a disease of silkworms that was well known in the 18th century. The problem of combating the disease was, therefore, given a great deal of attention late in the 18th century and particularly in the first half of the 19th century. The disease had been known for a long time in France as "muscardine de ver à soie" (because insects infected with the fungus were transformed into white mummified specimens that resembled comfits or bonbons) and in Italy as "calcino" (calcium or white powder) (131). Even now, the disease occasionally causes heavy losses in the rearing of silkworms. In Northern Italy in 1926, losses from the ravages of the calcino fungus were estimated at over 6,000,000 kgm. of cocoons valued at more than 150,000,000 lira (107).

It has become evident, however, that while *B. bassiana* and other species have often proved to be a costly pest to the sericultural industry they are, nevertheless, of some importance in the control of many noxious insect pests. Timonin (140) has shown *B. bassiana* to be a pathogen of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Steyaert (129) concluded that the introduction of *B. bassiana* into coffee-growing areas may be useful in the control of *Stephanoderis hampeii* Ferr. Some value has been attached to *B. densa* (Link) Picard in the control of cockchafers (*Melolontha* spp.) in damp forest soil rich in humus (19, 120). In general the frequent association of members of the genus *Beauveria* with insects throughout the world suggests that they may be important in the natural control of many insect pests.

That representatives of the genus *Beauveria* are not necessarily entomogenous was indicated by the isolation of *B.* (= *Sporotrichum*) *globulifera* (Speg.) Picard from the flesh of dead birds by Smith (124). Further evidence was produced by Kuru (62), who isolated *B.* (= *Isaria*) *shiotae* (Kuru) Langeron from an abscess located in the right hypochondrium of a human in Tokyo. He later found that the fungus showed a certain pathogenicity for guinea pigs and rats when inoculated into the abdominal cavity. Vincens (143) reported that *B. oryzae* Vincens was capable of causing a decay among the young roots of rice seedlings. *B. heimii* Saccas was isolated from paper in France by Saccas (117). There is now, however, some question as to whether the last two species should be placed in the genus *Beauveria*, since they resemble species of the genus *Tritirachium*, which is saprophytic rather than entomogenous.

The present investigation consists of three parts:

Taxonomic Review. *Beauveria* and *Tritirachium* species.

General Investigations on Criteria Used in Describing Members of the Genus *Beauveria*.

General Observations on Virulence.

It is hoped that these studies may lead to a clearer concept of the fungi associated with insects and serve as a basis for investigations concerning the physiology and histology of parasitism.

Taxonomic Review of *Beauveria* and *Tritirachium* Species

I. *BEAUVERIA* SPECIES AND THEIR TAXONOMIC VARIANTS

A. Beauveria bassiana (Bals.) Vuill., Bull. soc. botan. France, 59 : 34-40. 1912.

Syn. *Botrytis bassiana* Bals., Linnaea, 10 : 609. 1835.
Botrytis necans, Massee, Kew Bull. 159. 1914. (See Petch (97).)
Botrytis stephanoderis Bally, Mededeel. Koffiebessenboekfonds, 6 : 1-147. 1923. (See Friederichs *et al.* (45).)
Beauveria laxa Petch, Trans. Brit. Mycol. Soc. 16 : 55-75. 1931.

Beauveria bassiana was originally described by Beauverie (9, 10) and Vuillemin (145) as follows: hyphae slender, 1.5–2 μ in diameter, hyaline, septate; colonies flat, mealy, or finely pulverulent with a chalky appearance somewhat like the surface of a newly broken piece of chalk, white to pale cream on the surface, does not color undersurface of potato dextrose agar; phialides, variable in shape, ventricose to filamentous, develop on the main hyphal branches or on short branchlets at right angles to the main axis, branching may be repeated, forming compact globose heads; spores borne on zigzag sterigmata that extend from the tip of the phialides, globose, 2.4 μ in diameter.

The host range and geographical distribution of this fungus is reported in detail by Gosswald (49), Siemaszko (122), Petch (94), Steinhaus (131), Charles (27), and Rockwood (111, 112, 113).

In 1914, Massee (cited by Petch (97)) described a fungus isolated from *Brachartona catoxantha* under the name of *Botrytis necans*. In his original description, Massee described the conidia as very numerous, acrogenous, solitary, globose, and 4 μ in diameter. Petch (97), who examined a type specimen of this species in the Kew Herbarium, concluded that the name was synonymous with *B. bassiana*. He reported that the conidia were globose, 1.5–2 μ in diameter, or broadly oval, 1.5 $\times$ 2 μ, as in a typical *Beauveria* culture.

According to Petch (95), Friederichs (45) described *Botrytis stephanoderis* on the coffee berry borer, *Stephanoderis hampei* Ferr. of Java. In 1926, Petch (95) transferred this fungus to the genus *Beauveria*. Its identification is, according to Bally, based mainly on quantitative rather than qualitative differences. *Beauveria stephanoderis*, for example, grew more luxuriously in culture than did *B. bassiana*, formed larger heads of spores, and produced coremia regularly in potato dextrose agar. Some of the sporogenous cells also gave rise to more than one spore-bearing filament.

Petch (95), Averna-Sacca (3), and Siemaszko (122) concluded that this fungus is synonymous with *B. bassiana*.

Beauveria laxa was isolated by Petch (97) from Lepidopterous larvae and cocoons, and from spiders collected from Ceylon. In his original description, Petch (97) stated that this fungus differs from *B. bassiana* in its more regularly globose vesicles and its irregular conidia. The conidia are situated close together on the sterigma, which is not easily discerned, as it, with its attached spores, generally separates from the sporogenous cell as a whole, simulating a chain of conidia. (In other *Beauveria* species the conidia are more widely separated and many of them fall off individually, so that for both reasons the sterigma is more easily seen.) The spore clusters also appear more compact than in other species of *Beauveria*. On Quaker oat agar, *B. laxa* fills the culture tube with a white, loose, fluffy mass of mycelium and spreads over the glass in strands. Conidia are produced somewhat tardily and in small scattered masses over the mycelium and not in a uniform stratum as in *B. bassiana*.

Siemaszko (122) concluded that *B. laxa* is a strain of *B. bassiana* since, in his opinion, the irregular shape of the spores is due to crowding within the compact spore clusters.

B. *Beauveria globulifera* (Speg.) Picard, Ann. école nat. agr. Montpellier, N.S. 13 : 121-248. 1914.

Syn. *Sporotrichum globuliferum* Spegazzini, Ann. Soc. Cient. Argentina, 10 : 278-285. 1880.

Botrytis acridiorum Brongniart and Delacroix, Soc. nat. agr. France, 1891.

Botrytis delacroixii Saccardo, Sylloge Fungorum, 10 : 540. 1892.

Isaria vexans Pettit, Bull. 97, Cornell Univ. Agr. Expt. Sta. Botan. and Entomol. Div. 399. 1895.

Botrytis effusa Beauv., Rapp. Comm. Admin. du Lab. d'études de la Soie, Lyon, 14. 1911.

Beauveria doryphorae Poisson and Patay, Comptes rend. 200 : 961-963. 1935.

In 1880, Spegazzini (128) isolated a fungus from *Monocropedium* sp. and from *Naupactus zanthographus* in the Argentine, and named it *Sporotrichum globuliferum*. In 1891, Thaxter, according to Siemaszko (122), established in pure culture a white fungus isolated from the chinch bug in the United States and classified it temporarily as *S. globuliferum*.

According to Petch (95), *S. globuliferum* was introduced from North America into Algeria in 1892, and distributed for the purpose of destroying *Haltica ampelophaga*. No appreciable results were observed in the following three years, but, in 1896, a fungus was isolated from a *Haltica* species and believed to be the introduced American species. Subsequently, it was introduced into France from Algeria without any immediate results. During 1911–1912, however, a fungus epidemic occurred on *H. ampelophaga* in the district into which the Algerian material had been introduced. This species was again identified as *S. globuliferum* Speg.

In 1914, Picard (102) published a paper on entomogenous fungi, in which he transferred Spegazzini's species to the genus *Beauveria* as *B. globulifera* (Speg.) Picard. Petch (95), however, has expressed doubt as to whether the French *B. globulifera* is the same as Pettit's (see *Isaria vexans*) and whether either of them is biologically *S. globuliferum* Speg. This uncertainty is believed to be due to the known variation in the color produced in the substrata by *B. globulifera* isolates. For example, some of the substrata colors are: yellow (78, 101), dirty yellow (34), greenish yellow (104, 117), purple (100), wine-purple (122), and no apparent color (64, 102, 122). Because of this variation, Siemaszko (122) suggested that *B. globulifera* may be a cosmopolitan species consisting of numerous biological strains, some of which undoubtedly existed in France before the American strain was brought from Algeria.

The distinguishing characteristic on which this species was erected is that the mycelium appears as a loose, floccose or cottony, white to creamy mass that gives rise to a pulverulent layer of cream-colored spores (34, 64, 66, 78, 95, 102, 108, 122, 128, 131).

Delacroix together with Brongniart (21) described, in 1891, a fungus species on the migratory locust of Algeria as *Botrytis acridiorum*. Saccardo (115) renamed it *Botrytis delacroixii* because the name *B. acridiorum* had already been pre-empted by Trabut. Petch (95) recognized the fungus as a representative of the genus *Beauveria* and called it *B. delacroixii*. Siemaszko (122) treated it as a strain of *Beauveria globulifera*.

The description of the fungus, according to Delacroix (33), is as follows: culture fluffy, does not color potato slabs, but produces a very pale pink color on clear gelatinized media that at times is scarcely visible and disappears after 10 days; spores globose, 2–2.5 μ in diameter.

Isaria vexans was isolated by Pettit (100) from a *Lachnosterna* species. Petch (95) concluded that it was the coremial form of *B. globulifera* and Siemaszko (122) that it was the form that colors potato wine-purple.

Pettit found that on potato agar the fungus develops a loose, cottony growth over the entire surface. Later the colony becomes densely covered with a farinaceous, creamy-white layer of spores. It also colors potato a distinct purple. After 26 days, the mycelial mass gradually collapses and 3 to 15 cylindrical structures are produced in its place. These develop into long (sometimes $2\frac{1}{2}$ cm. in length), slender, erect, and usually clavate structures, generally simple, though sometimes branched. Pettit concluded that the development of coremia or mycelial aggregates was diagnostic of the genus *Isaria*.

In 1911, Beauverie (9) named another fungus isolated from the silkworm *Botrytis effusa*. In 1912, following Vuillemin's (145) erection of the genus *Beauveria*, this species was included as *B. effusa*. Siemaszko, however (122) concluded that the fungus was the same as the *B. globulifera* form that colored potato slabs a wine-purple.

This fungus is responsible for a disease of the silkworm called the red muscardine (9) because of the dark red color that it gives to infected larvae. Its identity was based on the purplish-red color that it gives to the reverse side of potato dextrose agar, and also because of the characteristic reddish color assumed by the surface of the colony after several days' growth.

Beauveria doryphorae was isolated from a number of dead potato beetles, *Leptinotarsa decemlineata* (Say), found in collections gathered near Rennes, France (104). Siemaszko (122) concluded that this fungus is identical with the *B. globulifera* form that does not color potato. Its main characteristic, according to Poisson and Patay (104) and to Patay (91), is the fact that it does not form coremia or produce any apparent color on Sabouraud maltose agar, blood agar, beef-broth agar, and potato agar.

C. *Beauveria tenella* (Delacr.) Siem., Archiwum Nauk Biologicznych Naukewego Warszawskiego, 6 : 1-83. 1937.

Syn. *Botrytis tenella* Delacr., J. agr. prat. 2 : 125-129; 163-167; 188-192; 1891.

Isaria densa (Link) Fries, Giard, Bull. sci. Nord France et Belg. 24 : 1-112. 1892.

Beauveria densa (Link) Picard, Ann. école nat. agr. Montpellier, N.S. 13 : 121-248. 1914.

Botrytis brongniartii Sacc., Sylloge Fungorum, 10 : 540. 1892.

In general *Beauveria tenella* has been described by previous investigators (33, 34, 48, 95, 100, 101, 122) as follows: colonies floccose, fluffy, cottony or mealy; surface color white through cream to yellowish; turns some infected insects pinkish to wine-red and the underside of gelatine an intense red to a deep vinaceous-purple, some isolates turn potato slabs varying shades of purplish-red, others from no apparent color to cinnamon-buff; hyphae 1.5–2.0 μ in diameter, hyaline, septate; sporogenous cells variable in shape, ventricose to filamentous, develop on the main hyphal branches and/or short branchlets at right angles to the main axis, forming compact globose heads. Spores oval to ellipsoidal, 2.0–6.0 μ × 1.5–3.0 μ, borne on zigzag, filamentous structures that extend from the tips of the sporogenous cells.

The host range and distribution of this fungus is reported in detail by Petch (97, 98, 99), Siemaszko (122), Gosswald (49), and Blunck (19).

This fungus is now commonly known as *Beauveria densa*. According to Prillieux and Delacroix (105), it is closely related to *B. bassiana* (Bals.) Vuill., but differs in that the spores are oval to ellipsoidal, whereas those of *B. bassiana* are globose (Plate I, Figs. 2 and 3).

Giard (48) did an extensive study of this fungus and he traced the synonymy as follows: In 1809, Link (70) described a fungus that he called *Sporotrichum densum*, but in this description he did not mention the size and shape of the spores. The same fungus was described and illustrated by Nees (86), in 1816, and the spores were reported to be round. In his monograph on the genus *Sporotrichum* published in 1820, Link (71) also described the spores as being round, and again called the fungus *S. densum*.

In 1822, Persoon (cited by Giard (48)) gave the name *Racodium entomogenum* to the vegetative stage of a fungus the spores of which were identical with those of *S. densum* Link.

In 1869, De Bary (31) found cockchafers infected with a fungus that he identified as *Botrytis bassiana* Bals. This fungus also had round spores like those of *S. densum* Link.

In 1886, Saccardo (114) described an *Isaria*-like fungus from cockchafers which he called *Botrytis tenella*. The spores were round, 1.5 μ in diameter, and he concluded that it was a variety of *Botrytis bassiana* Bals.

In 1891, Prillieux and Delacroix (105) applied the name *Botrytis tenella* to a fungus in which the spores were oval to elliptical, but which otherwise was close to *Botrytis bassiana* Bals.

In his study, in 1892, Giard (48) referred to the fungus as *Isaria densa* (Link) Fries. He assumed that the psuedocoremia (hyphasmates), which usually occurred on the cockchafer grubs, were the true coremial structures that are characteristic of the genus *Isaria*. The name *I. densa* he ascribed to Fries (46), but in the reference quoted as the authority, Fries did not mention the name *I. densa*. Rather, he applied the name *Sporotrichum densum* to *Botrytis densa* Ditmar (*Tricholuma candidum* Alb. et Schwein) but concluded that this was not *S. densum* described by Link. Since there does not appear to be any case where Fries has applied the name *I. densa* to a fungus, it must be concluded that Giard was in error.

In his original description of the genus *Beauveria*, Vuillemin (145) included two species only, *B. bassiana* and *B. effusa*. He expressed doubt with regards to *S. densum* Link (= *I. densa* Giard; = *Botrytis tenella* Sacc.) and its relationship to the genus *Beauveria*.

In 1914, Picard (102) gave the name *Beauveria densa* (Link) to Giard's fungus, but otherwise accepted Giard's nomenclature without reservation.

Siemaszko (122) concluded that *Sporotrichum densum* Link must be regarded as an anomalous species because of its incomplete description (70). For that reason he questioned whether *S. densum* could be considered as a synonym for the *Beauveria* species with oval spores, and therefore proposed that *Beauveria tenella* (Delacr.) should be the name of the fungus, since it is the first valid name in chronological order.

January 1st, 1821*, has been arbitrarily fixed as the date for the beginning of mycological nomenclature; thus *Sporotrichum densum* Link is not valid. This name was applied, in 1832, by Fries (46) to a different fungus and under the existing rules (Botanical Conference, Stockholm, 1950) it is valid for that fungus, making *Sporotrichum densum* Link a homonym and illegitimate for the *Beauveria* species with the elliptical spores. Therefore, the correct name for the fungus in question, with authorities, is *Beauveria tenella* (Delacr.) Siem.

Beauveria brongniartii was isolated from locusts in Algeria by Brongniart (21) as a *Botrytis* sp. In 1892, Saccardo (115) referred to it as *Botrytis brongniartii*. He reported that the spores were oval in shape, 4 × 2–2.5 μ

* *Art. 20. (F). International Rules of Botanical Nomenclature. 1947.*

in size, and slightly longer than the spores of *B. tenella* Delacr. Delacroix (33) also reported that this fungus differed from *B. tenella* in that it did not color gelatine red (*B. tenella* gives it a red color). Petch (95) transferred this species to the genus *Beauveria* as *B. brongniartii* in 1926. Because of the characteristic size and shape of the spores, Siemaszko (122) concluded that *Beauveria brongniartii* is a strain of *B. tenella*.

D. Beauveria peteloti Vincens, Bull. soc. botan. France, 61-62 : 132-143. 1915.

Beauveria peteloti was described and named by Vincens (142) in 1915 from three collections from Tropical America, one on the wasp *Polybia chrysothorax*, another on the wasp *Polystes canadensis*, and the third on bees, *Apis* sp. This fungus is considered by Petch (97) not to be a *Beauveria* species but close to *Hirsutella saussurei* (see Observations I, B).

E. Beauveria oryzae Vincens, Rev. pathol. végétale et entomol. agr. France, 10 : 112-131. 1923.

This species was one of several molds isolated from sclerotia of the fungus *Sclerotium oryzae*, which, according to Vincens (143), is believed to be responsible in Cochin-China for some of the rice diseases confused under the name "tiem". Under this species Vincens included two forms "a" and "b", the characteristics of which are as follows:

Form "a". . . . . Colony a compact, cottony, mycelial mass composed of filaments, 1–2 μ in diameter; white in color; conidiophores up to 1 mm. in length, 2 μ in diameter; develop fertile branches that may exist individually, more generally in whorls of 2 or 3, fertile branches, 10–20 $\mu \times$ 1–2 μ, each tapering near the apex to form a slender and flexuous dentate axis, 0.5 μ in diameter, and sometimes 20–30 μ in length. Twenty or more spores sometimes borne successively on the thread-like or filamentous apical portion, spores globose or almost globose, 1.3–1.5 μ, rarely 2 μ, in diameter.

Form "b" differs from form "a" in that the colony consists of a velvety mass, at first white, later becoming pinkish or ochre-like. The fertile branches also appear to be more rigid and more differentiated. They are about 20–25 μ in length, and 1.5–2 μ in diameter at the base, tapering at the apex in a slender dentate axis not as long or flexuous as that of form "a". The spores are a light pink in color, ellipsoid or oval 1.5 $\times$ 2 μ.

F. Beauveria paranense (Speg.) Gosswald, Arb. biol. Anst. (Reichsanst.) Berlin, 22 : 399-452. 1939.

Syn. *Sporotrichum globuliferum* Spegazzini., Ann. soc. cient. Argentina, 10 : 278-285. 1880.

Sporotrichum paranense Marchionatto, Bol. mens. min. agr. nacl. Buenos Aires, 34 : 227-245. 1933.

Sporotrichum paranense is stated by Marchionatto (76) to have been originally detected in 1897 by L. Bruner, while investigating the winged locust, *Schistocerca paranensis* Burm., in the area surrounding the town of Caracana.

Bruner recognized it as a *Sporotrichum* but was unable to furnish a specific name. Specimens submitted to Spegazzini were identified as *Sporotrichum globuliferum*, a determination that was later shown to be incorrect (75, 76). The importance of this fungus in controlling the winged locust has been pointed out in numerous publications (44, 75, 77, 106). A fungus believed to be the same as the Argentinian *S. paranense* was also reported on the locust, *Nomadacris septemfasciata*, in South Africa (82). Gosswald (49) listed *S. paranense* as a species of the genus *Beauveria*.

G. Beauveria rileyi (*Farlow*) *Gosswald*, Arb. biol. Anst. (Reichsanst.) Berlin, 22 : 399-452. 1939.

Syn. *Botrytis rileyi* Farlow, Riley, C. V., U.S.D.A. Report. 121. 1883.
Spicaria prasina Petch, Trans. Brit. Mycol. Soc. 11 : 258-266. 1926.
Beauveria bassiana Averna-Sacca, Bol. agr. Sao Paulo, 31 : 10-24; 195-213. 1930 (Abstr. Rev. Appl. Mycol. 10 : 188. 1931).

This species was first described by Farlow in 1883 as *Botrytis rileyi* in a report on the cabbage *Plusia* issued by Riley (110). This fungus was also isolated from the velvet-bean caterpillar (146), the clover looper (29), and the green clover worm (55). It was also isolated from the coffee berry beetle borer by Averna-Sacca (3), who identified it as *Beauveria bassiana*.

Petch (96) believed that the description of the fungus approached more closely that of a *Spicaria*, particularly the species *S. prasina* (Maubl.) Saw. Charles (25) concluded that, since the spores were described as borne in whorls and catenulate, the fungus could not be referred properly to the genus *Botrytis*. In view, however, of its close morphological affinities with the genus *Spicaria*, and the priority of the specific name, she concluded that the fungus should be designated *Spicaria rileyi* (Farl.) Charles. In 1939 Gosswald (49) listed this fungus as *Beauveria rileyi*.

H. Beauveria coccospora (Charles) Linder, Lloydia, 5 : 206. 1942.

Syn. *Rhinotrichum* (?) *depauperatum* Charles, Mycologia, 32 : 540. 1940.

This fungus was observed by Charles (26) on spider mites (*Paratetranychus yothers*) of the common water hyacinth (*Piaropus crassipes*) and designated *R. depauperatum*.

Charles acknowledged that this fungus closely resembled the fungus *R. album* described by Petch (96). At the same time, however, she described the fungus as a new species simply because of the uncertainty associated with Petch's description of the species *R. album* (see *B. coccorum*). Linder (69), in his account of this species, concluded that the spores are produced on reduced phialides, which are arranged in whorls, the phialides being represented by stout teeth that often in turn bear smaller teeth that give them a zigzag appearance. Since phialides with zigzag apices characterize the genus *Beauveria*, Linder proposed for this fungus the new combination *Beauveria coccospora* (Charles) Linder.

I. Beauveria coccorum (Petch) Linder, Lloydia, 5 : 206. 1942.
Syn. *Rhinotrichum* (?) *album* Petch, Trans. Brit. Mycol Soc. 11 : 258-266. 1926.
Gonatorrhodiella coccorum Petch, Trans. Brit. Mycol Soc. 16 : 55-75. 1931.

The fungus *Rhinotrichum album* was isolated from *Lecanium hemisphaericum* and described by Petch (96). In 1931 Petch (97) stated that further collections of this species and their culture on Quaker oat agar have shown that it is the same as *Gonatorrhodiella coccorum* found on black *Aleyrodes* on Mango (93). In his reference, however, to the synonymy of *R. album* and *G. coccorum*, Petch did not publish an amended description, thus failing to account for the character differences in the original description. Linder (69) concluded that *R. album*, reduced to synonymy under *G. coccorum*, could be placed in this genus only by extending the generic limits to a point where the genus *Gonatorrhodiella* would have no meaning, and proposed that it be placed in the genus *Beauveria* as *B. coccorum*.

J. Beauveria heimii Saccas, Rev. Mycol. N.S. 13 : 61-81. 1948.

This species was isolated from a packing box located in a cellar at Juan Gulf, France, in August, 1947, and on a book from the public library at Thiveral, France, in December, 1947. A description of this species is deferred to Section I, B, under observations, because of obvious incorrect generic disposition.

II. *TRITIRACHIUM* SPECIES AND THEIR TAXONOMIC VARIANTS

Various species of *Beauveria* have been confused with other genera ever since the genus was named and described by Vuillemin (145) in 1912. Some of them have been briefly mentioned, for example, *Botrytis*, *Isaria*, and *Sporotrichum*. These can be distinguished from *Beauveria* on the basis of certain morphological characteristics specific to each.

Limber (68), who named and described the genus *Tritirachium* in 1940, based its identity on the same characteristic that Vuillemin had used to establish the genus *Beauveria*. Limber stated that species of *Tritirachium* are readily distinguished from other forms of *Moniliaceae* by the zigzag appearance of the fertile parts of the conidiophores. This particular characteristic was originally described by Beauverie (9) in *Beauveria* (= *Botrytis*) *bassiana* and *B.* (= *Botrytis*) *effusa*; thus when Vuillemin described the genus *Beauveria* his description was based essentially on this characteristic spore-bearing filament.

Despite the similarity in the method of spore production, representatives of *Tritirachium* and *Beauveria* can be differentiated on the basis of other characteristics. The same degree of importance, however, has not been attached to these characteristics by different investigators. Consequently, species of *Beauveria* have been confused with the genus *Tritirachium*, and conversely species of *Tritirachium* have been treated as species of *Beauveria*. The

problem now is whether representatives of *Tritirachium* should be transferred to the genus *Beauveria* or be permitted to remain as species of the newer genus.

Apparently Limber was not the first to discover the type of fungus on which he erected the genus *Tritirachium*. According to him a Hyphomycete described by Ferraris (42), in 1912, under the name of *Sporotrichum flavicans* Fries var. *spicatum* closely resembled his species.

Beyma (13) reported that a similar fungus was also encountered in Japan by Saito in 1939, who described it under the name *Spirotrichum purpureum*. A pure culture of *S. purpureum* was later sent to the Centraal Bureau Voor Schimmelcultures, where Beyma recognized its similarity to an alleged *Verticillium* species (that he later identified as *S. musae*) isolated from banana leaves in Suriname by Stahel in 1934. Unfortunately Saito's description was in Japanese and, since a translation was not available, Beyma in 1940 published his interpretation of the genus on the basis of the two species *S. purpureum* and *S. musae*. Later when Beyma reviewed Limber's paper on the genus *Tritirachium*, he readily observed that Limber's descriptions and illustrations of the species applied to those of the genus *Spirotrichum*. Limber's almost simultaneous publication (68) with that of Saito's (cited by 13) referring to one and the same genus of fungus raised the question of priority and therewith the proper name to select for the genus in accordance with international rules. Beyma (14) concluded that since a Latin description had not been published by Saito for *Spirotrichum* this name should be superseded by Limber's and its species be transferred to *Tritirachium*, with *Spirotrichum purpureum* Saito becoming *Tritirachium purpureum* (Saito) Beyma, and *Spirotrichum musae* Beyma becoming *Tritirachium musae* (Beyma) Beyma.

Beyma in his treatise stated that *Beauveria* and *Tritirachium* should not be treated as synonyms. He felt that despite the characteristically identical manner in which the spores are formed in both cases, there are other differences of sufficient importance to warrant their being kept separate. In *Tritirachium*, the primary, secondary, and tertiary branches that occur successively along the central axis of the conidiophores, as well as the spore-bearing elements that develop at the extremity of the outer branches and at the tip of the conidiophores, are disposed in whorls; in *Beauveria*, the sporogenous cells may occur individually, in twos, or in groups along the main axis of the mycelium, or on conidiophores, which in turn may be simple or branched, occurring individually, in twos, or in thick rosettes, seldom in whorls, along the hyphae to form the sporiferous clusters typical of *Beauveria* species. Moreover, in their cultural habits, according to Beyma, *Tritirachium* species develop a very limited growth, which is bush-like in appearance, whereas *Beauveria* species spread out rapidly on artificial media, forming a more or less procumbent to flocculent mycelial growth with a powdery conidial surface.

Langeron (65) also treated *Beauveria* and *Tritirachium* as separate genera. He stressed that the conidiophores and phialides in *Tritirachium* were larger than those of *Beauveria* species. He concluded by transferring the following species from *Beauveria* to *Tritirachium: epigaeum*, *rubrum*, *shiotae*, *viannai*, and *brumpti*.

Saccas (117) isolated a fungus from a packing-box cover located in a cellar at Juan Gulf, France, and described it in 1948 under the name of *Beauveria heimii*. According to Saccas this fungus is very similar to the species described by Limber under *Tritirachium*, except perhaps for one or two minor characteristics. Saccas emphasized the fact that the phialides in the genus *Beauveria* end in a zigzag filament carrying conidia alternatively, and since this is the manner in which spores are formed in *Tritirachium*, he concluded that the form genus *Tritirachium* was identical with that of *Beauveria*, and transferred Limber's three species to *Beauveria* as *Beauveria dependens* (Limber) Saccas, *B. alba* (Limber) Saccas, and *Beauveria spicata* (Ferr.) Saccas. From the present study it was found that species characteristic of *Tritirachium* can be differentiated from *Beauveria* species. Moreover, it was found that some species described and named as members of *Beauveria*, e.g. *B. heimii* and *B. oryzae*, are more characteristic of the *Tritirachium* group.

A. Tritirachium dependens Limber, Mycologia, 32 : 23-30. 1940.
Syn. *Beauveria dependens* Saccas, Rev. Mycol. N.S. 13 : 61-81. 1948.

B. Tritirachium album Limber, Mycologia, 32 : 23-30. 1940.
Syn. *Beauveria alba* Saccas, Rev. Mycol. N.S. 13 : 61-81. 1948.

C. Tritirachium spicatum (Ferraris) Limber, Mycologia, 32 : 23-30. 1940.
Syn. *Sporotrichum flavicans* Fries var. *spicatum* Ferraris, Ann. Mycol. 10 : 295. 1912.
Beauveria spicata Saccas, Rev. Mycol. N.S. 13 : 61-81. 1948.

Saccas (117) concluded that he was justified in transferring the above three species to the genus *Beauveria*, in view of the fact that they had a similar mode of spore production.

D. Tritirachium roseum Beyma, Antonie van Leeuwenhoek, 8 : 118-120. 1942.

This species is a typical *Tritirachium* species. Its conidiophores are apparently simple and resemble those of *T. album* and *T. brumpti*. For a description and illustration of this species see Beyma (14, p. 119).

E. Tritirachium musae (Beyma) Beyma, Antonie van Leeuwenhoek, 8 : 116-120. 1942.
Syn. *Spirotrichum musae* Beyma, Antonie van Leeuwenhoek, 6 : 286-288. 1940.

This species was isolated by Van Stahel from a banana leaf-spot at Suriname and sent to the Centraal Bureau voor Schimmelcultures in February, 1934, where it was cultured as an alleged *Verticillium* species for several years. In 1940, Beyma (13) named it *Spirotrichum musae* because of its resemblance to *S. purpureum*. Later, when *Spirotrichum* was superseded by *Tritirachium*, it became *Tritirachium musae* (14).

Tritirachium musae is a typical *Tritirachium* species. Its conidiophores are apparently simple and resemble those of *T. album*, *T. brumpti*, and *T. roseum*. For a description and illustration of this species see Beyma (13, p. 286).

F. Tritirachium purpureum (Saito) Beyma, Antonie van Leeuwenhoek, 8 : 116-120. 1942.

Syn. *Spirotrichum purpureum* Saito, see Beyma (14).

The original description of this fungus published in Japanese (14) was not obtainable. According to Beyma (14), this species was described, in 1939, by Saito, who named it *Spirotrichum purpureum*. This species, along with *Spirotrichum musae*, was later transferred to the genus *Tritirachium* when the genus name *Spirotrichum* was superseded by *Tritirachium*.

Beyma reported that *T. musae* and *T. purpureum* are very closely related and differ from each other only in color and manner of growth on artificial media (13, 14).

G. Tritirachium cinnamomeum Beyma, Antonie van Leeuwenhoek, 8 : 116-120. 1942.

This species is a typical *Tritirachium*. Its conidiophores are apparently complex and resemble those of *T. dependens*. For a description and illustration of this species see Beyma (14, p. 116).

H. Tritirachium brumpti (Langeron) Langeron, Ann. parasitol. 22 : 94-99. 1947.

Syn. *Beauveria brumpti* Langeron, Bull. acad. méd. Paris, 111 : 133-137. 1934.

This fungus was isolated from a case of Keratoconjunctivitis (cured by an intensive iodinated treatment) in a 17-year-old girl resident in Cairo. The fungus was isolated by Dr. Elie Lichaa, and described by Langeron (64), who called it *Beauveria brumpti*. Langeron placed this fungus in the genus *Beauveria* because of the presence of bottle-shaped phialides with terminal zigzag spore-bearing filaments, which he believed to be characteristic of *Beauveria*. It differed from *B. bassiana* by its floury colonies, always pink or red in color, never white, and from *B. densa*, *B. effusa*, and *B. globulifera*, in that it did not produce flocculent or cottony colonies on artificial media.

In a later paper, published in 1947, Langeron (65) concluded that morphologically *B. brumpti* resembled more closely the species *Tritirachium dependens*, and transferred it to *Tritirachium* as *T. brumpti* (Langeron) Langeron.

I. Tritirachium epigaeum (Aschieri) Langeron, Ann. parasitol. 22 : 94-99. 1947. Not Brunaud, Nouv. Fragm. Mycol. 2, Series, Bordeaux 1888; Saccardo, Syll. Fung. 10 : 534. 1892. (See Dodge (36).)

Syn. *Sporotrichum epigaeum* Aschieri, Atti ist. botan. univ. Pavia, 4 : 199-222. 1930.
Beauveria epigea Langeron, Brumpt, Précis de Parasitol., Paris, Mason et Cie, 1936.

A fungus, isolated from cutaneous and osseus lesions on the feet of a patient in Venice, was sent by Professor Fiocco for identification to the Laboratory of Cryptogamic Botany at the University of Pavia, Italy. There Aschieri (1) concluded that it was identical with a soil saprophyte described and named by Brunaud (cited by Dodge (36)) as *Sporotrichum epigaeum*. According to Brumpt (22), Langeron later transferred *S. epigaeum* to *Beauveria* and in 1947 (65) to *Tritirachium* as *T. epigaeum*.

It is apparent from Aschieri's publication, however, that he was not certain of the identity of this organism. According to him, the fungus presented certain characteristics that tended to relate it to representatives of the genus *Beauveria*. Dodge (36) also reported that the conidia develop solitarily or sympodially in small groups on short branches or in whorls, sometimes suggesting conditions seen in *Beauveria*.

J. *Tritirachium shiotae* (Kuru) Langeron, Ann. parasitol. 22 : 94-99. 1947.
Syn. *Isaria shiotae* Kuru, Japan. J. Med. Sci. 9, Surg. 2 : 327-358. 1932.
Beauveria shiotae Langeron, Brumpt, Précis de Parasitol., Paris, Mason et Cie, 1936.

Kuru (62) isolated *Isaria shiotae* from an abscess in the right hypochondrium of a patient in Japan. For a detailed description of this fungus (with illustrations) see Kuru (62, pp. 337-346). He reported that it was morphologically identical with *B. bassiana* and *B. effusa*, and expressed the opinion that this fungus could possibly be an entomogenous strain, which happened to infect a human. He, nevertheless, placed it in the genus *Isaria* because the colonies occasionally formed coremia.

Langeron, cited by Brumpt (22), recognized this fungus as a *Beauveria* species and transferred it to this genus, as *Beauveria shiotae*. In 1947 he again transferred it (65), this time to *Tritirachium*, as *T. shiotae*.

K. *Tritirachium rubrum* (Baquis) Langeron, Ann. parasitol. 22 : 94-99. 1947.
Syn. *Verticillium rubrum* Baquis, Ann. ottalmol, 34 : 945-947. 1905.
Beauveria rubra Langeron, Bull. acad. méd. Paris, 111 : 133-137. 1934.
Spicaria rubra Dodge, Medical Mycology, C. V. Mosby Co., St. Louis, U.S. 843. 1935.

This fungus was originally isolated and named *V. rubrum* by Baquis (6) in Italy from lesions in cornea keratomycosis in 1905.

Langeron (64) reported that this fungus has been insufficiently described, but at the same time transferred it to *Beauveria* in 1934, and to *Tritirachium* in 1947. Dodge (36) in his review on Medical Mycology placed it in the genus *Spicaria* in 1935.

L. *Tritirachium viannai* (Froilano de Mello) Langeron, Ann. parasitol. 22 : 94-99. 1947.

Syn. *Trichophyton viannai* Froilano de Mello, Indian J. Med. Research, 5 : 222-233. 1917.

Beauveria viannai Langeron, Bull. acad. méd. Paris, 111 : 133-137, 1934.

Ateleothylax viannai Dodge, Medical Mycology, C. V. Mosby Co., St. Louis, U.S., 431. 1935.

This fungus was isolated and named *Tritirachium viannai* by Froilano de Mello (47) from a soldier suffering from a dermatosis of some months' duration on the thighs, pubis, and anterior part of the abdomen. In 1934, Langeron (64) transferred this species to *Beauveria*, and then to *Tritirachium* in 1947 (65).

Dodge (36) considered this species to be a member of the genus *Ateleothylax*, because of the characteristic appearance of perithecia present throughout the culture. The "vrilles" which Langeron thought similar to the sporiferous structures of the genera *Beauveria* and *Tritirachium* are, according to Dodge, related to the "spiral hyphae" found in the genus *Ectotrichophyton*, which is believed to include the imperfect stage of some of the species of *Ateleothylax*.

Materials and Methods

The insects from which fungi were isolated were obtained either from collections sent directly to the Laboratory of Insect Pathology or from routine collections of the Forest Insect Survey of the Division of Forest Biology. In the latter case the insects had been collected in the field by forest biology rangers and co-operators and sent to the Laboratory in cardboard mailing tubes. At the laboratory the living insects were placed with fresh foliage in sterile jars. The jars were checked daily and any dead material was sent to the Laboratory of Insect Pathology as had been insects that were dead on arrival. The dead material was then checked for the presence of fungi.

Larvae were considered as fungus-infected when they showed indications of fungi growing on their surface or were hard and mummified. Isolations made from such infected larvae were species of the following genera: *Cephalosporium*, *Spicaria*, *Beauveria*, *Sorosporella*, *Hirsutella*, *Isaria*, *Fusarium*, *Entomophthora*, and *Empusa*. Emphasis was placed on the genus *Beauveria*, since species of this genus were most frequently isolated.

The mummified larvae were surface-sterilized in 0.01% mercuric chloride in 80% alcohol for two minutes, then rinsed in four washings of sterile distilled water. More recently, a 2–5% Javex (sodium hypochlorite) solution has been used for surface sterilization, since subsequent rinsing in water is then unnecessary. After sterilization, from one to eight specimens were placed on sterile nutrient agar (Sabouraud maltose agar) in Petri plates. Whole specimens were used in the majority of cases, since many of the fungi that did not grow readily on nutrient media generally developed on the body of the insect. Only one type of culture developed on the majority of specimens thus tested. On a few insects, a combination of fungi, or fungi and bacteria, occurred. In

these cases, pure cultures were established by transferring a small portion of the mycelium from each fungus directly to Sabouraud maltose agar slants. As soon as the fungi started to develop spores on the surface of the insect material and/or artificial media, they were examined microscopically for identification.

After 20 days, spores were selected at random from new subcultures and placed on five different microscope slides, from each of which 20 spores were measured. The spores and material for morphological study were mounted in lactophenol to which had been added 0.5% (36) Harleco Anilin Blue (Hartman and Leddon Co.). The spore measurements were made with a Bausch and Lomb movable scale eyepiece micrometer used in a Leitz "Ortholux" research microscope with an apochromatic oil-immersion (2 mm.) objective and 12× oculars. The scale was graduated to 5 mm. in 100 parts (0.05 mm.). Each division corresponded to 0.5 μ. The length and breadth of each spore was then measured to the nearest whole division or to 0.5 μ. Drawings were made with a Spencer camera lucida.

A comparative study was made of the vegetative growth, pigment formation on the surface and in the substratum, and spore production of each isolate by inoculating larvae of either the original host or *Pristiphora erichsonii* (Htg.). In some cases larvae of *Choristoneura fumiferana* (Clem.) or of Geometrids, e.g. *Lambdina fiscellaria fiscellaria* (Guen.), were used as the host.

The isolates were also established on seven types of nutrient media: blood agar base, corn meal agar, potato dextrose agar, and Sabouraud maltose agar (Difco), Molisch culture medium (127), Czapek-Dox medium (57), and Raulin-Thom medium (87).

The single-spore cultures were established by preparing a spore suspension in which the number of spores per milliliter was determined with a Levy-Hausser counting chamber. One milliliter of the spore suspension was then transferred to a sterile test-tube containing 9 ml. of sterile, double-distilled water and diluted until the final suspension contained 100–200 spores per ml. One milliliter was then placed on sterile nutrient medium in a Petri plate, and allowed to germinate at room temperature for 16–20 hr. The germinating spores, much larger than the original spores, were readily located by examining the surface of the medium under an "Ortholux" research microscope with an 8 mm. objective and 12× oculars. The area surrounding the spore was then marked with a single-spore isolator (63), after which the marked section with the single spore was transferred by a sterile microspatula to an agar slant.

The *Beauveria* representatives isolated in the present investigation were compared with cultures of *Beauveria* species obtained from the Centraal Bureau Voor Schimmelcultures at Baarn, Netherlands (referred to throughout as C.B.S.), the American Type Culture Collection at Washington, D.C. (referred to as A.T.C.C.); the Bureau of Plant Industry at Beltsville, Maryland; and the Laboratory of Cryptogamic Botany at the National Museum of Natural History in Paris, France.

Observations

I. MORPHOLOGY AND TAXONOMY

A. General Investigations on Criteria used in Describing Members of the Genus *Beauveria*

1. CULTURAL CHARACTERISTICS

(a) Gross Morphology

(i) Primary Growth

Petch (95) concluded that the growth characteristics of a given species of *Beauveria* are dependent on the type of medium employed; of nine *Beauveria* strains tested on seven different types of media only one retained the same growth characters on all media. Rockwood (111, 112, 113) infected living and killed alfalfa weevils with *B. bassiana*. The killed weevils then showed a long, thin, cottony growth of mycelium with scattered spore clusters; those that subsequently died showed the typical short, matted mycelium, thickly covered with a mealy coating of spore clusters. The length of the mycelial growth on insects and on cultures, even when the material is from the same original source, may thus vary considerably.

Lefebvre (66) reported that *B. bassiana* from the corn borer produced a characteristically flat, mealy, chalky, pulverulent growth on 15 types of media, whereas *B. globulifera* produced a characteristically elevated, cottony, loose, floccose mycelial growth on all the media except one.

Siemaszko (122, 123) found that *B. globulifera* strains originating from various Polish insects do not lose their ability to produce fluffy mycelium. He concluded, therefore, that the macroscopic appearance of the mycelium among *Beauveria* isolates is a satisfactory diagnostic characteristic.

Siemaszko was of the opinion that the flat, powdery appearance of *B. bassiana*, on the one hand, and the loose, elevated, cottony or woolly mycelial growth characteristic of *B. globulifera*, on the other, were due mainly

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FIG. 1. The characteristic gross morphology associated with strains of the species *B. bassiana*.

The isolates *A* to *E* inclusive are *B. bassiana* strains from the following insect species: *M. disstria*, *C. fumiferana*, an unknown insect sp. Ottawa, Ont., *L. fiscellaria*, and *C. cossus*. *A*, *C*, and *E* have been inoculated into *P. erichsonii*, *B* and *D* into their natural hosts. The flat appearance of *A*, *C*, and *E* is characteristic of *B. bassiana*. Note that the colonies do not completely conceal the appearance of the infected larvae. *B* and *D* are somewhat elevated.

The isolates *F* to *J* inclusive are *globulifera* strains of *B. bassiana* from the following insect species: *M. disstria*, *S. rufimitrana*, *N. swainei*, *Choristoneura pini*, and a *Geometrid*, respectively. *F* was inoculated into its natural host, *G* and *H* into *P. erichsonii*, *I* into *C. fumiferana*, and *J* into *L. fiscellaria*. The flocculent appearance of the cultures is characteristic of the *globulifera* strain. Note that the colonies completely mask the appearance of the infected larvae 0.7 ×.

FIG. 2. Normal spores from species of the genus *Beauveria*. Dark field photomicrograph of spores (globose in shape) from *B. bassiana* on *B. mori*. 1000 ×.

FIG. 3. Dark field photomicrograph of spores (oval in shape) from *B. tenella* received from C.B.S., Baarn, Holland. 1150 ×.

PLATE I

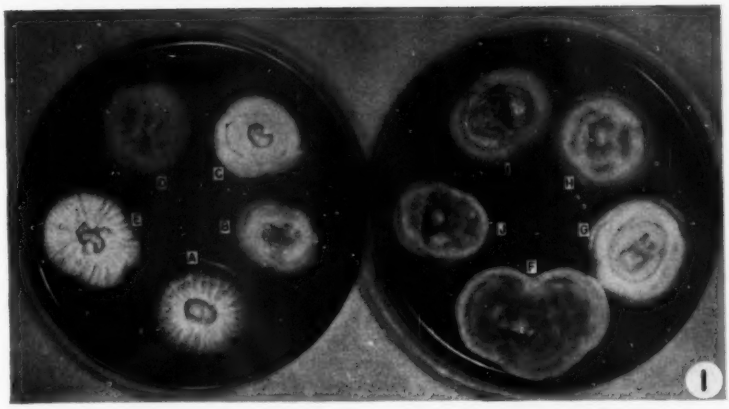

PLATE II

to the relative quantities of conidia and hyphae produced. The very rapid and abundant formation of spores by the *B. bassiana* strains covers the surface of the medium with a dusty coating that has the appearance of powdered chalk. The cultures of *B. globulifera*, as a result of copious mycelial development, retain their fluffiness for a considerable period of time (sometimes two or three weeks) and expand above the level of the nutrient. Following the production of spore masses, which in some strains takes place only after several weeks, the colonies become mealy, in some cases even dusty. When this happens, it is sometimes difficult to distinguish between old cultures of *B. bassiana* and *B. globulifera.*

In the present investigation it was found that the gross morphology (type and amount of growth and spore production) of the various isolates as they developed on naturally infected insect species showed slight variation. This was also true among cultures inoculated into their original host species. The mycelial growth was generally procumbent and showed extremely heavy spore-production. Occasionally, atypical growth occurred on naturally infected insect material, but more frequently in cases where cultures were inoculated into different insect species. For example, isolates from *Campaea perlata* Gn. and *Lambdina somniaria* (Hlst.), inoculated into *P. erichsonii* and *L. fiscellaria fiscellaria* respectively, produced mycelia that were elevated to flocculent, but cultures from *Epicnaptera americana* Harr. and *Semasia rufimitrana* H.-S. displayed poor to fair spore-production on *P. erichsonii.*

On artificial media the variation in cultural growth was much more apparent. On Sabouraud maltose agar, 17 isolates were typically *B. bassiana*, 15 typically *B. globulifera* (Plate I, Fig. 1), and 34 showed gradations between the characteristics of both species. Of the latter, 15 were regarded as *B. bassiana* and 19 as *B. globulifera.* There was also considerable variation in the quantity of spores produced by the cultures in each group. Another culture, obtained from C.B.S. as *B. globulifera*, proved to be identical with some of the cultures identified as *B. bassiana.* Cultures of *B. effusa* (Beauv.) Vuill. and *B. tenella* (Delacr.) Siem. from the same source were similar to some of the members of the species *B. globulifera.* It thus becomes a matter of the judgment of the

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FIGS. 1-4. Secondary growth of strains of *B. bassiana.*

FIG. 1. A culture of *globulifera* strain from *M. disstria* on Sabouraud maltose agar for four months. A large portion of the culture has been covered by secondary growth, sterile and whitish in color. The new growth is more or less flat and spreading in habit. .5 ×.

FIG. 2. A culture of *globulifera* strain from *C. fumiferana* on Sabouraud maltose agar for four months. The secondary growth has formed a loose cottony efflorescence over the surface of the culture. .7 ×.

FIG. 3. A culture of typical *B. bassiana* from *S. cylindricollis* on Sabouraud maltose agar after one month. Note that the surface of the culture is covered with long cylindrical shaped coremia. 1 ×.

FIG. 4. A larva of *C. pomonella* covered with a *B. bassiana* culture from *B. mori.* The coremia vary in size and shape. The large coremium in the center has formed a series of finger like projections near the apex, each of which has a bulbous tip. Note that some coremia are covered with spore clusters. 6 ×.

observer as to whether the cultures should be identified as *B. bassiana* or *B. globulifera*. On this basis cultures considered to be *B. bassiana* or *B. globulifera* were isolated from insect species as shown in Table I.

None of the isolates, when transferred to other types of media, retained the same gross morphology in all cultures. The *B. globulifera* cultures, when transferred from Sabouraud maltose agar to Molisch and Czapek-Dox media, showed a decrease in the number of isolates that developed typical *B. globulifera* characteristics. At the same time, there was an increase in the number of isolates resembling *B. bassiana* strains. On Raulin-Thom medium, none of the isolates displayed typical *B. globulifera* characteristics but all to a large extent resembled *B. bassiana* strains. On blood agar base, the change in characteristics was more pronounced, with some two-thirds of the isolates (65.7%) appearing as typical *B. bassiana* strains. The remainder, because of their extremely poor growth generally, were characteristic of neither species.

A culture identified as *B. stephanoderis* from C.B.S. was also included under *B. bassiana*. *Beauveria effusa* from the same source was included under *B. globulifera*. *Beauveria tenella* although resembling *B. globulifera* differed from it in the size and shape of its spores.

The *B. globulifera* isolate from *N. sertifer* that had lost its typical *B. globulifera* characteristics when transferred to Czapek-Dox medium and grew as a typical *B. bassiana* strain regained its flocculent growth when transferred back to Sabouraud maltose agar, thereby displaying the gross morphology of the original culture.

Spore production among the various *B. globulifera* isolates also varied considerably. It was heaviest on blood agar base, followed by Sabouraud maltose agar and Molisch medium, Raulin-Thom and Czapek-Dox media, potato dextrose, and finally corn meal agar.

The variation among the *B. bassiana* isolates was much less spectacular. The extremely flocculent mycelial growth characteristic of *B. globulifera* cultures did not develop in any of the cultures, although two cultures on Molisch medium and one on Raulin-Thom medium resembled the less typical *B. globulifera* strains.

The heaviest spore-production among the *B. bassiana* isolates occurred on Sabouraud maltose agar, followed by Molisch medium, blood agar base, Raulin-Thom and Czapek-Dox media, potato dextrose, and finally corn meal agar.

Many of the isolates in both the *B. bassiana* and *B. globulifera* series showed a reduction in vigor on potato dextrose and corn meal agar, where growth and spore production in general were extremely poor. As a result no typical *B. bassiana* or *B. globulifera* cultures developed on such media.

Many insects are natural hosts of both *B. bassiana* and *B. globulifera* (see Table I). A series of naturally infected specimens of *C. fumiferana*, *M. disstria*, and *N. sertifer* also gave rise, when plated on Sabouraud maltose agar, to both *B. bassiana* and *B. globulifera* strains, as well as to cultures that displayed various gradations between the characteristics of the two species. Some of

TABLE I

INSECT SPECIES FROM WHICH *Beauveria* CULTURES HAVE BEEN STUDIED

Host	Family	Order	*B. bassiana*	*B. globulifera*
Melanoplus sp.	Locustidae	Orthop.	+*	−†
Camnula pellucida Scudd.	Acrididae	"	−	+
Sitona cylindricollis Fahr.	Curculionidae	Coleop.	+	−
Popillia japonica Newm.	Scarabaeidae	"	+	−
Scymnus impexus Muls.	Coccinellidae	"	−	+
Acanthocinus aedilis L.	Cerambycidae	"	−	+
Pyrausta nubilalis (Hbn.)	Pyralidae	Lepidop.	+	−
Crambus sp.	"	"	+	−
Mineola vaccinii (Riley)	"	"	−	+
Cacoecia murinana Hbn.	Tortricidae	"	+	−
Choristoneura fumiferana (Clem.)	"	"	+	+
Choristoneura pinus Free.	"	"	−	+
Archips fervidana Clem.	"	"	−	+
Archips cerasivorana (Fitch)	"	"	−	+
Unidentified. sp.	"	"	−	+
Bombyx mori L.	Bombycidae	"	+	−
Campaea perlata Gn.	Geometridae	"	+	−
Lambdina somniaria Hlst.	"	"	+	−
Nepytia canosaria Wlk.	"	"	+	+
Oporinia autumnata Gn.	"	"	+	−
Venusia cambrica Curt.	"	"	+	−
Enyptia venata Grt.	"	"	−	+
Unidentified sp.	"	"	−	+
Malacosoma disstria Hbn.	Lasiocampidae	"	+	+
Malacosoma pluviale (Dyar)	"	"	−	+
Malacosoma americanum (F.)	"	"	−	+
Tolype sp.	"	"	+	−
Epicnaptera americana Harr.	Lasiocampidae	"	−	+
Carpocapsa pomonella (L.)	Olethreutidae	"	+	+
Sciaphila duplex Wlshm.	"	"	+	−
Unidentified sp.	Phalaenidae	"	+	−
Zenobia pleonectusa Grt.	"	"	−	+
Cossus cossus L.	Cossidae	"	+	−
Unidentified sp.	Liparidae	"	−	+
Euphydryas chalcedona (Dbldy. and Hew.)	Nymphalidae	"	−	+
Nymphalis antiopa (L.)	"	"	−	+
Hyphantria textor Harr.	Arctiidae	"	−	+
Semasia rufimitrana H.-S.	Eucosmidae	"	−	+
Paraclemensia acerifoliella Fitch.	Incurvariidae	"	−	+
Monoctenus juniperinus MacG.	Diprionidae	Hymenop.	+	−
Neodiprion abietis (Harr.)	"	"	+	−
Neodiprion banksianae Roh.	"	"	+	−
Neodiprion lecontei (Fitch)	"	"	−	+
Neodiprion sertifer (Geoff.)	"	"	+	+
Neodiprion swainei Midd.	"	"	−	+
Neodiprion tsugae Midd.	"	"	+	−
Neodiprion virginiana Roh.	"	"	+	+
Diprion hercyniae (Htg.)	"	"	−	+
Hemichroa crocea (Fourc.)	Tenthredinidae	"	+	−
Pikonema alaskensis (Roh.)	"	"	+	−
Pristiphora erichsonii (Htg.)	"	"	+	+
Unidentified sp.	"	"	+	−
Trichiosoma triangulum Kby.	Cimbicidae	"	−	+

* + = isolate present.
† − = isolate absent.

these are heavy spore-producers, others are sterile, and still others show atypical vegetative growth with poor to fair spore-production. It was possible to separate 205 isolates selected at random from naturally infected *N. sertifer* larvae into at least 55 distinct groups, when the different shades of color were considered with the variations in gross morphology. These cultures were grown on Sabouraud maltose agar under the same environmental conditions.

In the course of eight generations of single-spore transfers, comprising 408 single-spore cultures, five variants were isolated from the *B. globulifera* strain on *N. sertifer*. The variants differed from the original parent-strain in gross morphology, color on the surface, and coloration in the substratum.

The characteristics of the isolates as they occurred on Sabouraud maltose agar are shown in Table II.

TABLE II

INTRASPECIFIC VARIATION AMONG ISOLATES THROUGH SINGLE SPORE CULTURING

Isolates	Growth*	Spore production†	Surface color‡	"Reverse"	Remarks
Parent	3 +	+ + +	White – pale cream $\left(Y\ \frac{3}{1}\right)$ with tinge of brown	Dark green $\left(G\ \frac{3}{1}\right)$	Rough, powdery with age, *B. globulifera*
Variant 1	3 +	+ + +	White – pale cream $\left(Y\ \frac{3}{1}\right)$ with tinge of brown	Red $\left(R\ \frac{5}{3}\right)$	Rough, powdery with age, *B. globulifera*
" 2	3 + – 4 +	+ +	Yellow red $\left(YR\ \frac{8}{4}\right)$	" $\left(R\ \frac{4}{4}\right)$	Somewhat powdery with age, *B. globulifera*
" 3	4 +	+	" " "	" $\left(R\ \frac{4}{4}\right)$	Loose, cottony appearance with age, *B. globulifera*
" 4	2 + – 3 +	+	" " "	" $\left(R\ \frac{5}{5}\right)$	Star-shaped fissure in substratum, intermediate between *B. bassiana* and *B. globulifera*
" 5	2 +	+ + +	Yellowish $\left(Y\ \frac{8}{4}\right)$	Yellowish $\left(Y\ \frac{8}{4}\right)$	Flat, procumbent, resembles *B. bassiana*

* *Amount of Growth: 2+, all of slant covered, 3+, all of slant covered, tube partially filled over slant, 4+, tube over slant filled with mycelium.*

† *Spore production: + poor, ++ fair, +++ good or abundant.*

‡ *Color designation taken from Munsell charts (Munsell, (84)).*

A *B. globulifera* isolate from *A. fervidana*—growth extremely flocculent (4+); spore production abundant (+++); surface color yellowish, close to $Y\ \frac{5}{6}$, but tinged with red; reverse side yellow-red $\left(YR\ \frac{5}{6}\right)$—gave rise, after two generations of single-spore transfers, to an isolate identical with Variant 4.

A *B. bassiana* culture from *N. sertifer*—growth flat or procumbent (2+); spore production heavy (+++); surface color yellowish $\left(Y\ \frac{8}{4}\right)$; reverse side yellowish $\left(Y\ \frac{8}{4}\right)$—also gave rise to an isolate identical with Variant 4 after one generation of single-spore transfers.

Thus three *Beauveria* isolates that differed in their cultural characteristics each gave rise in the course of one or more generations of single-spore cultures to identical variants.

Occasionally *Beauveria* cultures in Petri plates became contaminated with a heavy spore-producing *Penicillium* species. If such cultures were sterile strains, the area of the *Beauveria* colony adjacent to the contaminant usually produced spores, particularly in cases where the contaminant was in actual contact with the *Beauveria* colony. This suggests that the *Beauveria* isolates were possibly obtaining from the contaminant some element or elements essential for spore production.

(*ii*) *Secondary Growth*

Secondary growth occurred on a number of the *Beauveria* isolates and existed in two forms: (*a*) mycelial blooms and (*b*) coremia.

(*a*) *Mycelial blooms.*—On some media, sterile mycelial growth consistently developed on the surface of cultures of *Beauveria* species after they had completed their normal mycelial and spore development. The new growth occurred either as a flat and spreading layer or as a loose, cottony efflorescence over the surface of the culture (Plate II, Figs. 1 and 2). The mycelial blooms always developed on *Beauveria* cultures established on infected insect larvae, Molisch medium, and Sabouraud maltose agar, which promote a rich vegetative growth, but did not occur on potato dextrose agar, corn meal agar, blood agar base, and other media where the vegetative growth was much less abundant. They also developed occasionally on Raulin-Thom and Czapek-Dox media. The mycelial blooms usually occurred on cultures established on Sabouraud maltose agar for two months or longer but sometimes developed in 17 days. They were always sterile and may have been mycelial mutants formed as a result of the drying-out of the medium or from some unknown factor. Similar observations have been made by Petch (95) and by Siemaszko (122).

(*b*) *Coremia.*—Occasionally on infected insects and artificial media, *Beauveria* isolates produced aggregates of mycelial fascicles called coremia, sometimes referred to as hyphasmates (48), clavules (10, 34), or psuedocoremia (122). These structures have been responsible for some of the confusion associated with *B. densa* found on *Melolontha vulgaris* L. throughout Europe. On some of the diseased specimens the fungus was flat and spreading, while on others it produced aggregated mycelial forms or coremia, which are more or less similar to the isarioid forms of the genus *Isaria*, and for this reason the fungus has been regarded as a member of the genus *Isaria*.

Coremia were frequently observed in *B. globulifera* cultures but not in *B. bassiana* cultures isolated from Polish insects (122). Among Canadian isolates they developed on cultures of both *B. globulifera* and *B. bassiana*. They were extremely variable in form (Plate II, Figs. 3 and 4). Some have been reported to be 1 to 2 mm. in length (34). Among Canadian isolates they ranged up to 60 mm. in length; some were thread-like, cylindrical, or clavate in shape, others branched; some formed very loose structures, others were close-knit; some were sterile, others extremely fertile. Occasionally both sterile and fertile coremia developed on the same isolate. They usually formed in old cultures (two to four months) but occasionally developed on some cultures after a week, on others after 17 to 21 days.

Coremial development was dependent to some extent on the type of medium used. Coremia did not grow on potato-dextrose agar, corn meal agar, blood agar base, and Raulin-Thom medium. They did, however, appear from time to time on infected larvae and on such media as Sabouraud maltose agar, Molisch, and Czapek-Dox media. But even then, coremia did not always develop on transfers from those cultures most consistently associated with coremial growth.

Conditions that bring about the aggregated forms of the fungus are for the most part not well known. Giard (48) has suggested that the absence of light may be an important factor. Picard (102) and Siemaszko (122) have stated that under conditions of considerable moisture, e.g. under bark or underground, the fungus grows at the expense of the infected larvae, developing the aggregated hyphae in sufficient length to branch all round the mummified larvae in the soil. Later on, the fungus may obtain substances from the organic matter of the soil and live as a saprophyte. Rockwood (113) has suggested that in nature the location of the insect at the time of death from fungus infection has an important influence on coremial development. He found that specimens in concealed locations, such as clover-root borers in mines in the roots, produced a longer mycelium, more often in the form of coremia, than did exposed specimens. Others believe that coremial formation in culture is due to some physiological variation resulting from the medium.

(*b*) *Color*

(*i*) *Surface Color*

Petch (95) has reported that a strain of *B. bassiana* that originally produced white spore masses in culture formed spore masses that were pale cream after a period of nine months. Siemaszko (122) concluded that surface color is not important in the distinction of species.

In this study it was found that the mycelium is invariably white at first, but as growth continues the color of the mycelium in some of the cultures gradually changes to resemble that of the spores, which usually form over the surface of the cultures as they mature. Some of the *Beauveria* isolates, as they develop

on insects over a period of three weeks, produce white spore masses, others show varying shades of yellow, ranging from Y $\frac{8}{1}$ to Y $\frac{7}{5}$, and still others are a darker yellowish-red, ranging from YR $\frac{8}{5}$ to YR $\frac{6}{4}$.

Some variation in color developed when the isolates were transferred to different types of media. In general, however, the colors consistently fell within a narrow range of the colors white, yellow, and a light brown or fawn. For example, on most of the artificial media (see Materials and Methods) tested, *B. stephanoderis* and *B. effusa* were white, but on blood agar base the former was yellowish $\left(\text{Y } \frac{7}{3}\right)$, whereas the latter on Molisch medium and potato-dextrose agar varied from Y $\frac{8}{2}$ to Y $\frac{8}{3}$. Isolates from a *Crambus* sp. and *N. canosaria* were yellowish $\left(\text{Y } \frac{8}{1}\text{–Y } \frac{8}{5}\right)$ on all media, those from *L. somniaria* and a *Tolype* sp. were yellowish $\left(\text{Y } \frac{8}{2}\text{–Y } \frac{8}{5}\right)$ on blood agar base, potato-dextrose agar, corn meal agar, Czapek-Dox medium, and Raulin-Thom medium, and a yellowish-red $\left(\text{YR } \frac{7}{4}\text{–YR } \frac{6}{3}\right)$ on Molisch medium and Sabouraud maltose agar. On the last, some of the cultures that varied from white to yellow on most of the media may show a faint tinge of red, e.g. an isolate from Liparidae; others that vary from white through yellow to yellowish-red, a faint tinge of orange, e.g. isolates from *D. hercyniae* and *N. swainei*; and still others a faint tinge of green, e.g. an isolate from *E. venata*.

Variation in the surface color also occurred among cultures from the same insect species. This held particularly when isolates were taken from a large number of specimens. For example, isolates from specimens of *M. disstria*, *C. fumiferana*, and *N. sertifer* varied from white through yellow and cream to brown with, in some cases, a faint tinge of red or orange.

With increasing age the cultures often became darker and assumed various shades of grayish-white, yellowish-brown, or reddish-brown.

According to Mulder (83), the addition of copper ions (2.5 μgm. per 40 ml.) increases the surface color of cultures of *Aspergillus niger*. A similar quantity, however, added to Czapek-Dox medium in this investigation failed to influence the color of any of the *Beauveria* cultures.

(*ii*) *Color in the Substratum*

(*a*) *Cause.*—Siemaszko (122) has reported that in cultures of *B. globulifera* isolated from *Pentatoma rufipes*, which color potato-dextrose agar a deep red, bacteria are always present. However, as soon as this fungus was established in pure culture on potato-dextrose agar through monosporidial culturing, it lost the ability to color the substratum. On the other hand, an examination of the bacterial cultures showed the presence of several races or strains of bacteria, two of which colored potato red. Siemaszko, therefore,

concluded that the coloration of the substratum is not necessarily due to the fungus but rather to the accompanying bacteria, since insects infected with *Beauveria* species are invariably contaminated with bacteria as well.

One of the earliest references to the association of *Beauveria* strains and bacteria in infected insects, according to Masera (80), was by Perroncito (92), who discovered the presence of *Serratia marcescens* Bizio in silkworm larvae infected with *B. bassiana*. This association has also been mentioned by others (3, 131), who seem to think that in some way the bacteria are connected with the biology of the fungus, and that the red coloring displayed by the insect in the later stages of infection is caused by the presence of the red-pigmented bacterium.

A series of specimens from different insect species naturally infected with *Beauveria* isolates that produce pigments in Sabouraud maltose agar were examined for bacteria. The infected larvae were surface sterilized in 70% ethyl alcohol and aqueous Hyamine (1/1000) and crushed in sterile tubes of Difco nutrient broth. After 48 hr. a loopful of the broth was plated upon Difco nutrient agar. From these plates transfers were later established on Sabouraud maltose agar slants. Stained slides were made from the different cultures and examined for bacteria.

After 48 hr. the broth was found to be clear except for fungal mycelium. No cloudiness such as is typical of bacterial growth occurred. On examination stained slides prepared from the nutrient broth showed no signs of bacteria. Transfers from the plates established on Sabouraud maltose agar slants produced pigments in the substratum. These were also found to be free of bacteria.

It is concluded from this experiment that bacteria are not always associated with *Beauveria*-infected larvae, and that the pigments formed in diseased larvae and in inoculated artificial media are not due to bacteria but instead are caused by the fungus.

(*b*) *Color in infected larvae.*—A number of *Beauveria*-infected larvae are pink to wine-red in the later stages of infection. Beauverie (9) and Dieuzeide (34) have pointed out that *B. effusa* changes silkworm larvae to a more or less violet-red. Delacroix (32) and Siemaszko (122) found that *B. densa* turns cockchafer larvae pink to wine-red. Siemaszko (122), Steinhaus (131), and Bartlett *et al.* (7) have reported that *B. bassiana* changes *C. cossus*, *B. mori*, and *P. nubilalis* pinkish to red. Siemaszko (122) has also observed *B. globulifera* to have a similar effect on some Polish insects.

Some Canadian insects showed no apparent change in color when naturally infected with *Beauveria* species, e.g. *M. americanum*, *M. pluviale*, and *M. disstria*. Others turned red $\left(\mathrm{R}\,\frac{5}{4}\right)$, e.g. *C. pomonella* and *M. vaccinii*; some turned a light yellow $\left(\mathrm{Y}\,\frac{8}{2}\right)$, e.g. *M. juniperinus* and *P. erichsonii*; while a few turned yellowish-red $\left(\mathrm{YR}\,\frac{5}{3}\right)$, e.g. *N. banksianae* and *D. hercyniae*.

Beauveria isolates from different individuals of a species did not always give the same color when inoculated into *P. erichsonii* larvae. Thus some cultures from *S. duplex* and *N. sertifer* turned *P. erichsonii* larvae red, others turned them yellowish. Moreover, spores from the same culture did not always react in the same manner; for example, spores from isolates from *C. pellucida*, *E. venata*, *S. cylindricollis*, and *N. tsugae* turned some *P. erichsonii* larvae red and others yellow, while spores from a single *N. virginiana* larva had no apparent effect on the color of some *P. erichsonii* larvae, turned others red, and still others yellow.

(*c*) *Color in artificial media.*—On some media, *B. bassiana* is associated with a brilliant red color, while on potato-dextrose agar there is no apparent color development (91). On gelatin medium *B. bassiana* develops a maroon color (33). *B. densa* gives a vinaceous-purple tinge to potato-dextrose agar (122), and also colors gelatin red (101). *B. globulifera* is associated with a variety of colors on potato-dextrose agar. These include yellow (78, 101), a dirty yellow (34), a greenish-yellow (104, 117), a purplish color (100), and a reddish-purple (122).

The variation in the intensity of color is sometimes influenced by the medium. Siemaszko (122) reported that a *Beauveria* isolate from *C. pomonella* at first colored a medium consisting essentially of mineral elements red, then greenish, and finally a dark olive-green; on potato-dextrose agar it immediately formed a dark wine-purple color.

A *Beauveria* isolate from the ant, *Oecophylla smagradina*, when sowed on corn meal agar, failed to color the medium, but resowed after a prolonged period on the same medium began to color it red (95).

After a period of subculturing, *Beauveria* isolates gradually lose their ability to color media. *B. globulifera* from a number of Polish insects (122) turned potato agar a more or less deep reddish-purple for a period of six months to a year; thereafter the color began to fade. *B. bassiana* and *B. densa* (48) can, according to the circumstances, either color or not color the substratum.

No importance was attributed to the color of the substratum by Siemaszko (122) in distinguishing *Beauveria* species isolated from a number of insect species. He believed, however, that color in the medium indicated forms within a species. On this basis he divided *B. globulifera* into two forms, one that turns potato agar a wine-purple and the other which does not.

Giard (48), Petch (95), and Picard (102) have reported that a loss of the power of imparting color to the substratum coincides with a loss of virulence by the fungus as a parasite on insects. Dieuzeide (34) failed to observe such a correlation, although Siemaszko (122) found that usually, when the ability to color decreases, the germinating ability of the conidia also weakens.

Numerous investigators (10, 34, 91, 104, and 117) have attempted to use the color produced by *Beauveria* cultures in artificial media as a means of separating species. It is evident from the literature, however, that color in the substratum is of no great importance in the determination of species.

The *Beauveria* isolates studied in the present investigation produced a wide range of colors in the substratum (sometimes designated color of reverse, or simply "reverse") after a period of three weeks. The colors included varying shades of yellow $\left(\text{Y}\ \frac{7}{4}-\text{Y}\frac{8}{4}\right)$, yellow-red $\left(\text{YR}\ \frac{7}{4}-\text{YR}\frac{5}{3}\right)$, green-yellow $\left(\text{GY}\frac{6}{3}\right)$, red $\left(\text{R}\ \frac{4}{6}-\text{R}\ \frac{2}{1}\right)$, green $\left(\text{G}\ \frac{4}{2}-\text{G}\frac{3}{4}\right)$, and blue-green $\left(\text{BG}\ \frac{3}{2}-\text{BG}\frac{2}{1}\right)$. In general the coloring was intense and showed considerable variation in Sabouraud maltose agar, Molisch medium, and blood agar base. Some isolates that produced varying shades of purple-red $\left(\text{R}\ \frac{2}{1}-\text{R}\frac{3}{7}\right)$, e.g. from *C. perlata* and *S. impexus* on Sabouraud maltose agar and Molisch medium respectively, developed a bright yellow color $\left(\text{Y}\ \frac{8}{4}\right)$ on blood agar base. Occasionally cultures, e.g. from a *Tolype* species, that produced a light yellowish-red $\left(\text{YR}\ \frac{5}{6}\right)$ in Sabouraud maltose agar and Molisch medium formed a darker red $\left(\text{R}\ \frac{2}{1}\right)$ in blood agar base.

Potato dextrose agar and Raulin-Thom medium were each colored by two isolates, Czapek-Dox medium by five, whereas none of the isolates formed a pigment on corn meal agar.

The yellow color produced by isolates from *Melanoplus* sp., *C. murinana*, *P. nubilalis*, and numerous others, on Sabouraud maltose agar was not influenced by subculturing over a period of time. The only possible exception was an isolate from *C. fumiferana* in which the color ranged from brownish-yellow to bright yellow. Several of the isolates from *N. tsugae*, *Tolype* sp., *S. duplex*, and others, which develop dark colors in the substratum, were also fairly constant. Numerous others showed considerable variation when subcultured over periods of 4 to 12 months. Isolates from *O. autumnata*, *C. perlata*, and Phalaenidae that originally produced a dark purplish-red $\left(\text{R}\ \frac{2}{1}\right)$ developed a lighter color or $\left(\text{YR}\ \frac{5}{6}\right)$ at the end of four months and a bright yellow $\left(\text{Y}\ \frac{8}{4}\right)$ within a year. An isolate from *C. pomonella* varied from purplish-red $\left(\text{R}\ \frac{2}{1}\right)$ through cherry-red $\left(\text{R}\ \frac{3}{7}\right)$ to a pale red $\left(\text{YR}\ \frac{7}{4}\right)$; one from *N. virginiana*, originally light purplish-red $\left(\text{R}\ \frac{3}{7}\right)$ admixed with green, changed to yellow $\left(\text{Y}\ \frac{8}{4}\right)$; an isolate from *E. americana*, at first green-yellow $\left(\text{GY}\ \frac{6}{3}\right)$ at the base of the slant and yellow tinged with red at the tip, changed to bright yellow $\left(\text{Y}\ \frac{8}{4}\right)$ within a year. On the other hand, an isolate from *V. cambrica* Curt., originally a bright yellow blended with red $\left(\text{YR}\ \frac{7}{4}\right)$, developed a darker red $\left(\text{R}\ \frac{3}{7}\right)$ on Sabouraud maltose agar after a year.

The color assumed by the substratum is not necessarily the same for all specimens of an insect species naturally infected with this fungus. Thus isolates from *N. sertifer*, when transferred to Sabouraud maltose agar, developed a wide range of colors, including red, blue to almost black, green, red and green, and yellow. Isolates from *C. fumiferana* and *M. disstria* larvae developed red to orange, green, red and green, and yellow colors.

Some of the isolates from *M. disstria* that formed a green color on Sabouraud maltose agar, when passed through *P. erichsonii* larvae, developed the same characteristic green, while others formed a red color completely devoid of green. Some cultures originally green with perhaps a tinge of red at the top of the slant usually became red after four months. The red pigment may thus be present from the beginning but be masked by the green pigment, becoming evident only after the green pigment has disappeared.

The color of the spore masses among some isolates seems to be influenced by the pigment formation on the "reverse" but among others the colors are entirely different. For example, an isolate from *E. venata*, which at the end of 24 days had developed a yellowish-red near the center and a pale green along the periphery on the reverse side, displayed the same pattern on the surface of the colony. On the other hand, an isolate from *S. duplex* that produced a dark green pigment in the substratum was white on the surface.

Some rather interesting color changes occurred in the substratum as a result of a series of single-spore transfers of a *B. globulifera* isolate from *N. sertifer*. This culture always developed a dark green $\left(\text{G}\ \frac{3}{1}\right)$ pigment in Sabouraud maltose agar. After two generations of single-spore transfers, a variant developed which produced a red pigment $\left(\text{R}\ \frac{4}{4}\right)$ in the substratum. Following the third transfer, a variant developed from the red-pigmented strain which colored the substratum yellow $\left(\text{Y}\ \frac{8}{4}\right)$. After the fifth transfer, cultures developed from the yellow variant identical with the red parent-strain, and following the sixth transfer some of the red variants developed cultures that had reverted back to the color of the original parent-culture.

The most spectacular change occurred in the fifth generation. In the fourth generation, a single-spore culture was noted that appeared yellow both on the surface and in the substratum during the first stages of growth but later gradually turned green in the substratum and appeared identical with the original parent-culture. In the fifth generation, this culture gave rise to three single-spore cultures, which in the early stages of growth resembled the original parent-strain and then abruptly changed their mycelial character to develop a flat, or *B. bassiana*, type of growth. The new growth, which started out with a reddish color $\left(\text{R}\ \frac{7}{2}\right)$ on the surface, finally turned yellow $\left(\text{Y}\ \frac{8}{4}\right)$ in the marginal area. In the substratum the cultures varied from dark green $\left(\text{G}\ \frac{3}{1}\right)$ to bright green $\left(\text{G}\ \frac{5}{6}\right)$ to yellow $\left(\text{Y}\ \frac{8}{4}\right)$ and some red was

also evident. In the following generation, one culture gave rise to some isolates that were green in the substratum, others red, and still others yellow; the second and third cultures gave rise to yellow and red cultures only.

Weiss and Nord (147) reported that the addition of zinc ions (2 mgm./liter) to Czapek-Dox medium increased pigment production in the substratum by the fungus *Fusarium solani*. A similar quantity of zinc added to Czapek-Dox medium had no influence on pigment production among the *Beauveria* isolates.

(*d*) *The influence of pH on color.*—Among the *Beauveria* isolates studied, there was no apparent color development in the early stages of growth. However, after a period of 4 to 10 days, the substratum of some cultures became deeply colored. According to Siemaszko (122), as *Beauveria* cultures develop there is a corresponding decrease in pH, which promotes the secretion of coloring matter from the mycelium to the medium. He found a *B. globulifera* isolate from *P. brassicae* that developed an intense wine-purple color on potato agar and altered the pH from 6.0 to 4.5, while a *B. bassiana* strain from *C. pomonella* that did not color the medium changed the pH from 6.0 to 7.0.

The following test was conducted in an attempt to determine if an increase in acidity is correlated with color production in the substratum among *Beauveria* cultures isolated from Canadian insects.

Five sets of six cultures each were prepared with Sabouraud maltose agar. Each of the first four sets was inoculated with an isolate from each of the following insect species: *N. canosaria* (I, red), *S. duplex* (II, red blended with green), unknown insect species from Ottawa, Ontario (III, no apparent color to yellowish), and *N. sertifer* (IV, bluish-green). The fifth set was left uninoculated as a control. The pH of the medium was taken just before the cultures were inoculated; thereafter, readings were taken from one tube of each set at intervals of five days for a period of 30 days. This was done by heating a culture from each set until thoroughly dissolved. The fungus mat was removed and the pH reading then taken at approximately 40° C. with a glass electrode.

It was found that the various isolates, regardless of the color produced, tended to increase the acidity of the medium. There appears to be no consistent relationship between the acidity and the depth of color. The cultures with the lightest and the heaviest pigment-production (III and IV respectively) showed growth inhibition after 10 days. At this point the pH readings reached their lowest point; thereafter, the curves flattened out. The cultures with intermediate pigment-production (I and II) grew increasingly acid over periods of 15 and 25 days respectively. The various cultures showed a slight rise in pH near the end of the test period.

(*e*) *Influence of light on color.*—Since it has been suggested (34) that light may have some effect on pigmentation, a test was set up to determine the effect of light on the color produced in the substratum by some of the *Beauveria* isolates.

Using Sabouraud maltose agar as the medium, three isolates from *N. sertifer* were each inoculated into 14 culture slants. The first isolate colors the substratum yellow, the second a blue-green, and the third red. Two cultures from each set were kept in the light, the remainder in complete darkness. Growth was at room temperature for 30 days.

It was clear that light does influence some pigments. The cultures grown in daylight showed slight pigmentation after 92 hr. This was also true of the cultures grown in complete darkness, with the exception of the series inoculated with the third or red pigment-producing isolate. This series showed no color in the substratum. After 30 days the cultures in the light were heavily pigmented. The yellow and green series in darkness were similarly pigmented, but no indication of the red color developed in the third series. After an incubation period of 10 days in darkness, three of the cultures from the third set were placed in the light. Shortly thereafter, a red color began to appear in the substratum.

It is thus evident that, while the yellow and green pigments will develop equally as well in light or darkness, the red pigment will develop in the substratum only in the presence of light.

2. DETAILS OF STRUCTURE

(*a*) *Spores*

(*i*) *Primary Spores*

In the species *B. tenella*, the spores are oval to ellipsoidal (33, 122, 124), and it was upon this characteristic that the species was finally described and named. The spores of *B. brongniartii* (115), *B. melolonthae* (116), and *B. shiotae* (62) are also oval and thus resemble those of *B. tenella*.

The spores of the remaining species in the genus, *B. bassiana* (3, 10, 30, 33, 34, 41, 66, 95, 97, 118, 122), *B. stephanoderis* (95, 122), *B. laxa* (97), *B. delacroixii* (33), *B. globulifera* (34, 66, 95, 108, 122), *B. effusa* (10, 34, 122), *B. vexans* (100), and *B. doryphorae* (104) are described as being globose. There are, however, several investigators (34, 66, 95, 97, 104, 128) who have referred to spores of *B. bassiana*, *B. doryphorae*, *B. stephanoderis*, *B. necans*, *B. laxa*, and *B. globulifera* as varying from globose to oval. There is also some inconsistency in the reported shape and dimensions of the spores of some *Beauveria* species (Table III).

In this study spore samples were taken from each of the following: *B. effusa*, *B. stephanoderis*, three isolates of *B. tenella* (received as "*B. densa*"), 31 isolates identified as *B. bassiana*, and 34 identified as *B. globulifera*. A sample of 100 spores was selected, since the results obtained with this number compared favorably with those from larger spore-samples (Table IV). The percentages of oval and globose spores in each species with their dimensions are shown in Table V.

On the basis of both size and shape, the spores of *Beauveria* species can be divided into two groups. The first includes the species *B. bassiana*, *B. globulifera*, *B. effusa*, and *B. stephanoderis*. In this group the spores are globose to

TABLE III

REPORTED SHAPE AND DIMENSIONS OF SPORES OF VARIOUS SPECIES OF *Beauveria*

Species	Size and shape of spores according to various authors
B. bassiana	Averna-Sacca (3) globose 2.5–4.0 μ
" "	Beauverie (10) globose 3.0–4.5 μ
" "	De Bary (31) globose 2.5–2.8 μ
" "	Delacroix (33) globose 2.0–2.5 μ
" "	Dieuzeide (34) globose 1.0–5.3 μ
" "	Ferraris (41) globose 1.5–3.0 μ
" "	Siemaszko (122) globose 1.9–3.3 μ
" "	Massee (cited by Petch 97) globose 4.0 μ
" "	Sawada (118) globose to oval
" "	Lefebvre (66) globose (2.1–2.5 μ) oval 1.0–6.0 μ × 1.0–4.0 μ
" "	Petch (95) globose (1.5 μ) oval 1.5–2.0 μ × 1.2–2.0 μ
" "	Petch (97) globose (1.5–2.0 μ) oval 1.5 × 2.0 μ
" "	Timonin (140) oval 2.0–3.5 μ × 2.0–3.0 μ
B. stephanoderis	Siemaszko (122) globose 1.7–2.8 μ
"	Petch (95) globose (1.2–2.5 μ) oval 2–2.5 μ × 1.5–2.0 μ
B. laxa	Petch (97) globose (1.5 μ) oval 1.5–2 μ × 1–1.5 μ
B. delacroixii	Delacroix (33) globose 2.0–2.5 μ
B. globulifers	Dieuzeide (34) globose 2.0–5.0 μ
" "	Reyes (108) globose 2.2 μ
" "	Siemaszko (122) globose 1.7–2.5 μ
" "	Lefebvre (66) globose (2.1–2.5 μ) oval 1.0–6.0 μ × 1.0–4.0 μ
" "	Petch (95) globose (2.0–2.5 μ) oval 2–2.5 μ × 1.5–2.0 μ
" "	Spegazzini (128) oval 2–2.5 μ × 1.5–2.0 μ
B. effusa	Beauverie (10) globose 2.0–4.0 μ
" "	Dieuzeide (34) globose 2.0–4.0 μ
" "	Siemaszko (122) globose 1.7–2.8 μ
B. vexans	Pettit (100) globose 1.7–2.5 μ
B. doryphorae	Poisson and Patay (104) globose to oval 1.8–2.0 μ
" "	Delacroix (33) oval 2.5–3.0 μ × 1.5–2.0 μ
B. tenella	Siemaszko (122) oval 1.9–5.0 μ × 1.7–3.0 μ
" "	Smith (124) oval 2.0–2.5 μ × 1.5–2.0 μ
B. brongniartii	Saccardo (115) oval 4.0 μ × 2–2.5 μ
B. melolonthae	Saccardo (116) oval 2.5–3.0 μ × 1.5 μ
B. shiotae	Kuru (62) oval 2.5–4.0 μ × 2.0–3.5μ

oval (Plate I, Fig. 2), with 2 μ being the largest single length- or diameter-class and 1.5 μ the largest single width-class. The second group includes the species *B. tenella* in which the spores are oval to ellipsoidal (Plate I, Fig. 3), with the largest number falling in the length-class 3 μ and the width-class 2 μ. Two per cent of the spores in this species were, however, globose, which suggests that *B. tenella* is not necessarily homogeneous for spore shape and may under certain conditions give rise to globose spores.

The spore measurements of *B. stephanoderis* and *B. effusa* were found to differ somewhat from those of *B. bassiana* and *B. globulifera*, although no indication was found in the literature (3, 9, 10, 34, 45, 145) that significant

TABLE IV

COMPARATIVE MEASUREMENTS OF SPORES FROM *Beauveria* ISOLATES

Source of *Beauveria* isolates	*Beauveria* sp.	Total No. of spores	Oval spores													No. globose	Globose spores					
			No. oval	Number of spores in ½ μ classes													No. spores in ½ μ classes					
				Length							Width						Diameter					
				3	4	5	6	7	8	9	2	3	4	5	6		3	4	5	6	7	8
V. cambrica	*bass.*	100	30	2	22	5	1	—	—	—	2	22	5	1	—	70	10	41	19	—	—	—
C. pomonella	*glob.*	300	115	1	62	37	10	4	1	—	1	62	39	13	—	185	11	83	84	5	1	1
Unidentified sp.*	*bass.*	600	225	—	71	130	16	6	2	—	1	113	103	7	1	375	48	237	88	1	1	—
C. fumiferana	*glob.*	900	404	2	157	171	53	16	2	3	2	183	157	62	—	496	15	178	262	41	—	—
M. disstria	*glob.*	1400	623	1	272	295	51	4	—	—	1	283	304	35	—	777	12	285	442	36	1	1

* *From Ottawa, Ontario.*

TABLE V

SUMMARIZED COMPARATIVE MEASUREMENTS OF SPORES FROM CULTURES OF *Beauveria* SPECIES

Beauveria sp.	Total No. of spores	% oval	Oval spores															% globose	Globose spores					
			% of spores in ½ μ classes																% of spores in ½ μ classes					
			Length										Width						Diameter					
			3	4	5	6	7	8	9	10	11	12	2	3	4	5	6	7		2	3	4	5	6	7
bassiana (31 isolates)	3100	42.8	0.1	20.4	18.1	3.6	0.5	0.1	—	—	—	—	0.2	22.6	17.3	2.7	—	—	57.2	—	3.2	30.9	22.3	0.8	—
globulifera (34 isolates)	3400	43.8	0.6	20.1	16.6	5.2	0.9	0.3	0.1	—	—	—	0.7	22.6	16.7	3.8	—	—	56.2	0.1	6.7	26.8	21.1	1.4	0.1
stephanoderis (1 isolate)	100	77.0	21.0	50.0	6.0	—	—	—	—	—	—	—	38.0	36.0	3.0	—	—	—	23.0	—	20.0	3.0	—	—	—
effusa (1 isolate)	100	79.0	—	2.0	24.0	39.0	10.0	3.0	1.0	—	—	—	1.0	2.0	32.0	41.0	3.0	—	21.0	—	—	4.0	15.0	2.0	—
tenella (3 isolates)	300	98.0	—	0.7	14.7	41.7	25.3	9.0	3.3	2.3	0.3	0.7	1.7	32.7	38.7	23.3	1.3	0.3	2.0	—	—	—	1.7	0.3	—

differences exist among the spores of these species. Indeed, in a recent publication (122), *B. stephanoderis* has been described as a strain of *B. bassiana*, and *B. effusa* as a strain of *B. globulifera*. The dissimilarity observed in this study between the spores of *B. stephanoderis* and *B. effusa*, on the one hand, and *B. globulifera* and *B. bassiana*, on the other, may therefore have been due to prolonged subculturing of both *B. effusa* and *B. stephanoderis*.

The frequency of globose spores varied considerably among both the *B. bassiana* and *B. globulifera* cultures. This range was from 31 to 70% in the 31 isolates of *B. bassiana* from different naturally-infected insects, and from 34 to 74% in the isolates of *B. globulifera*, also from different naturally-infected insects. The difference, however, was not entirely due to dissimilarity among the insect species source, since 66% of the spores were globose in a *B. bassiana* culture isolated from a larva of *N. canosaria*, while only 34% were globose in a *B. globulifera* culture taken from the same insect species. On the other hand, when a single culture of *B. bassiana* isolated from a *B. mori* larva was used to infect 19 insect species in nine different orders, the development of globose spores in these cultures varied from 48 to 81%.

Beauveria species formed spores on infected insects 48 hr. after the mycelium first appeared on the integument. On artificial media, however, spore production was slower and varied somewhat among the different species. For example, spore development among some *B. bassiana* cultures required 53 hr. from the start of incubation. With *B. tenella* and *B. stephanoderis*, spore production started after 63 hr.; *B. globulifera* was slower, the first spores not appearing until four to five days. Lefebvre (66) reported that in *B. bassiana* abundant spore production took place within a week, usually in three or four days; in *B. globulifera* under identical conditions, spore formation did not occur until the cultures had been growing for two weeks to a month and then only sparsely.

(ii) Secondary Spores

De Bary (31) found that, when primary spores from cultures of *Beauveria* species were germinated in a thin film of water, the germ tubes, after reaching a length of 6 to 10 times the diameter of the spores, produced conidia that differed from the normal conidia. They lacked separate pedicels, were free on the apex of the sterigmata, and dispersed readily; they were not globose but oblong-cylindric about the same diameter as, or slightly narrower than, the normal conidia and three to four times as long. De Bary named these conidia "cylinder conidia". After the formation of cylinder conidia, the hyphae continued their growth and produced normal heads of globose spores.

Secondary spores were also reported among *Beauveria* species by Pettit (100) and Siemaszko (122). The latter found that in drop cultures (4% sugar solution) secondary spores appeared on a typical *B. bassiana* strain from C.B.S. after 24 hr., usually on hyphae not exceeding 10–12 μ in length. In a *B. globulifera* culture isolated from *Ips typographus* (L.) and in one from a *Leptura* species, the secondary conidia appeared after 36–48 hr. incubation,

on hyphae 75–100 μ in length. Among other *B. bassiana*, *B. globulifera*, and *B. densa* strains, Siemaszko noted variation in the number, size, and shape of secondary spores formed in this way. The secondary spores were oval or cylindrical and ranged in size from 7–12 $\mu \times 3$–3.5 μ.

Among the Canadian strains similar secondary spores were occasionally produced when primary spores of both *B. bassiana* and *B. globulifera* germinated directly on sterile microscope slides, without media of any kind, at 25–28° C. and 95–100% R.H. Secondary spores also occurred in some of the cultures. The number of cylindrical or irregular spores seemed to be associated with the age of the culture. They were not observed on new cultures, but after periods of two to four months or more, they occasionally developed in fairly large numbers. The secondary spores usually occurred directly on the mycelium (Text-fig. 1, Figs. 2, 3, 4, 10, 11, 12) or on hypha-like sporogenous cells, sometimes with normal spores (Text-fig. 1, Figs. 1, 5, 7); they rarely developed on normal sporogenous cells, but in the few cases where this occurred, they formed a small cluster at the tip of the cell (Text-fig. 1, Fig. 6) or formed in small numbers among a group of normal spores (Text-fig. 1, Figs. 8, 9). The variation in the size and shape of spores removed from some cultures that had been established on Sabouraud maltose agar for six months is shown in Text-fig. 1, Figs. 13-15.

(*iii*) *Germinating Spores*

Germinating spores among the Canadian isolates were studied by examining, at regular intervals, spores that had been sown on Sabouraud maltose agar in sterile Petri plates.

Spores from strains of *B. bassiana*, *B. stephanoderis*, *B. globulifera*, *B. effusa*, and *B. tenella* germinated in eight hours at room temperature. The spores at first swelled and then produced one to three protuberances at any place on the spore, although germination generally took place either at the tip or near the base (Text-fig. 2, Figs. 1, 2, 13). The germ tubes were thin-walled, hyaline, continuous, and cylindrical in form; a few ranged up to 15 μ in length at eight hours (Text-fig. 2, Figs. 6, 9, 10). At 16 hr.,[1] they ranged from a few microns to as much as 60 μ in length (Text-fig. 2, Figs. 4, 7, 8), with one or two cross-walls present in some of the longer tubes (Text-fig. 2, Figs. 3, 5, 13).

Secondary branches started to form at 20 hr. (Text-fig. 2, Fig. 12) and four hours later they occurred in large numbers, branching off at an angle of 45° to 90° from the main germ tube (Text-fig. 2, Figs. 2, 11). The beginning of the first tertiary branch was observed at 24 hr. (Text-fig. 2, Fig. 14). Thereafter, the number of both secondary and tertiary branches rapidly increased, forming a loose mycelial network over the surface of the medium. At 36 hr. no essential differences were evident among any of the species; later, however, the aerial growth that formed on the cultures of *B. effusa* and *B. tenella*

[1] *The time intervals mentioned in the discussion include the period of incubation.*

resulted in the flocculent or woolly vegetative growth characteristic of *B. globulifera*. On the *B. stephanoderis* cultures the vegetative growth was flat or procumbent, typical of *B. bassiana*.

(*b*) *Mycelium*

Among the *Beauveria* isolates, the mycelial strands consist of cylindrical, frequently anastomosing hyphae, in which the cross-walls are more or less closely spaced, depending on the age of the culture. In immature cultures, the cross-walls occur at intervals of 25.5 to 53.0 μ; in older cultures, they are much more numerous and occur at intervals of 7.5 to 15.5 μ. The diameter of the immature filaments is approximately 1.5 μ, increasing to 2.0 and occasionally to 3.5 μ as they mature.

(*c*) *Conidiophores*

When spores are produced abundantly by *Beauveria* isolates, they are borne in rather compact, globose heads, either on the main hyphal branches or on short laterals, referred to as conidiophores (Text-fig. 3, Figs. 12, 13). These develop from the mycelium at right angles near the septa and appear at first as tumor-like projections, which gradually enlarge to form hypha-like elements on the main hyphae (10, 95, 145).

The conidiophores show considerable variation in both size and shape. Some of the typical ones are simple, others branched. The unbranched ones are oblong, varying from almost globose to practically cylindrical, 2.5–5.5 μ $\times$ 1.5–2.5 μ (Text-fig. 3, Fig. 2); cylindrical, 3.0–28.0 μ $\times$ 1.5–2.5 μ (Text-fig. 3, Figs. 3, 12, 13, and 15); and flask-shaped, 4.0–15.5 μ $\times$ 1.0–2.0 μ (Text-fig. 3, Figs. 4 to 8). The branched conidiophores vary, 15.5–25.5 μ $\times$ 1.5–3.0 μ (Text-fig. 3, Figs. 14 and 16). Some of the extremely long conidiophores form a sinuous mycelial type of structure (Text-fig. 3, Fig. 18).

The atypical conidiophores are sometimes narrow or wide, bent or curved, and vary in size, 6.0–19.5 μ $\times$ 1.0–3.5 μ (Text-fig. 3, Figs. 9, 10, 17, 19, 23). These were observed infrequently and are not specific to any one isolate.

The sporogenous cells (see following section) that give rise to spores are sometimes borne directly on the conidiophores but usually on spherical-shaped cells, so-called prophialides (40, 140, 144), associated with the conidiophores. In this paper, however, it is proposed to refer to these cells as vesicles, since it is believed that the term prophialide is unsuitable, as the spherical cells do not give rise to true phialides.

The intermediate cells, or vesicles, may first appear as globose cells directly on the mycelium and/or at the tip and along the sides of the conidiophores (Text-fig. 3, Figs. 12 and 13). At maturity they give rise to sporogenous cells, or secondary vesicles, or both. The secondary vesicles in turn give rise to another series of vesicles and sporogenous cells. This process may be repeated several times until, in the final stages of development, there exists a more or less rounded, compact cluster of vesicles, sporogenous cells, and spores.

The vesicles generally range in diameter from 1.5 μ to 5.0 μ, with an average of 2.5 μ. Occasionally within spore clusters that are very compact, the vesicles may be irregular in shape and vary from oval through oblong to more or less rectangular.

The variation in size and shape of the conidiophores and vesicles is influenced to some extent by the type of medium and the vitality of the fungus. It was found that these elements tended to disappear in some cultures that were subcultured for a prolonged period.

(*d*) *Sporogenous Cells*

Earlier investigators (10, 11, 34, 66, 91, 95, 117, 140, 145) have referred to the spore-bearing filaments in this genus as phialides. According to Hughes (58), however, a phialide is a cell that develops one or more open ends, from which a basipetal succession of conidia (phialospores) develop without an increase in the length of the phialide itself. The phialospores may form a slimy head or develop in chains that are unbranched by virtue of their basipetal development. Since spores are not formed in this group in this manner, the spore-bearing filaments are apparently not true phialides, and will be treated simply as sporogenous cells. The spores will be referred to as terminal spores (81), because each spore terminates the growth of the spore-bearing filament, further growth taking place by the formation of a new growing-point below the apex.

The sporogenous cells occur singly, in pairs, or in small clusters, sometimes directly on the mycelium, but more frequently on vesicles within the typical *Beauveria* clusters.

It has been pointed out by Vuillemin (145), Beauverie (10), Petch (95), and Siemaszko (122) that the spore-bearing filaments display considerable variability in size and shape; they may be ventricose, oblong, fairly short and slender, or elongate, or thread-like to the point where they resemble vegetative filaments. These authors found that the sporogenous cells may occur directly on the mycelium, particularly when growth takes place in an unfavorable environment, but more frequently on short branchlets or vesicles. They concluded that in spite of the variation noted among the spore-bearing filaments, one characteristic that remains constant is the particularly interesting process by which the sporogenous cells develop spores at their tips (see following section).

Although the sporogenous cells are single-celled, the basal section, or body-region, and the tip, or thread-like filamentous section, have been treated separately in this study in order to show more clearly the variation in morphology associated with both parts.

(*i*) *The Basal Section*

The basal section includes the region of the body up to, but not including, the point at which the first spore is produced. This section displays considerable variability in both size and shape. The typical sporogenous cells are

generally globose, varying in diameter from 1.5–3.5 μ (Text-fig. 4, Figs. 1 to 8), with an average diameter of 2.5 μ. Some of these develop an irregular shape, possibly through crowding as the cluster becomes more compact, varying from 3.0–7.5 μ × 1.0–3.5 μ (Text-fig. 4, Figs. 9-11).

The atypical sporogenous cells generally occur directly on the mycelium and the conidiophores. These vary from cylindrical, 7.0–10.0 μ × 1.0–2.0 μ (Text-fig. 4, Figs. 12 to 14), to curved and flask-like, 7.5–15.0 μ × 1.0–3.0 μ (Text-fig. 4, Figs. 15, 17, to 20), to thread-like filaments, 7.5–28.0 μ × 1.0–1.5 μ, some of which are straight and erect (Text-fig. 4, Figs. 28 and 32), others curved (Text-fig. 4, Figs. 21 to 27). A few are extremely long, forming a sinuous mycelial type of sporogenous cell, 65 μ or more in length (Text-fig. 4, Fig. 29). Individual clusters borne directly on the mycelium contain sporogenous cells that are not always similar in size and shape (Text-fig. 4, Figs. 30, 31, and 33).

It is difficult to determine where the cylindrical, flask-shaped, and hypha-like sporogenous cells start to taper and continue their growth as thread-like filaments. This point, however, is much more evident among the globose sporogenous cells. In this group there is some variation in the distance between the body of the sporogenous cell and the first spore. This distance may vary from 0.5–5.0 μ (Text-fig. 4, Fig. 5) but in general is less than 1.0 μ. It was also observed that some of the sporogenous cells occur on a short, stalk-like base (Text-fig. 4, Fig. 4), less than 1.0 μ in length, though as a rule the sporogenous cells are sessile.

When *Beauveria* cultures are established on unsuitable media the basal portion of the sporogenous cells becomes irregular in shape and frequently forms the atypical, or filamentous, type difficult to distinguish from the mycelium, or it disappears entirely.

(*ii*) *The Terminal Section*

The terminal section consists of a very thin, thread-like filament, which extends from the body of the sporogenous cell and bears the spores.

The growth of the filament with subsequent spore-development is shown in Text-fig. 5, Figs. 1-12. The sporogenous cell at first forms a very thin thread, the tip of which becomes inflated to form a small, globular ampoule (Text-fig. 5, Fig. 1*a*), which rapidly develops into spore No. 1. This spore, while it does not hinder the elongation of the drawn-out portion of the sporogenous cell, does terminate the growth of the axis upon which it was borne. A new elongation of the axis, therefore, is initiated a little below the point where the spore is attached, its growth pushing aside spore No. 1 (Text-fig. 5, Fig. 2). The new axis, No. 2, forms at its extremity spore No. 2 (Text-fig. 5, Fig. 3, *b*). A new elongation of the axis is started as spore No. 2 is formed in a manner similar to that of the preceding one. Spore No. 2 is also pushed aside, while a third spore takes form at the summit of the third broken axis (Text-fig. 5, Fig. 4, *c*). This process is repeated a number of times (10–20) until there is a series of branchlets (occasionally referred to as sterigmata (43, 78. 114)) in

a zigzag line. At the tip of each branchlet, spores are formed on short, sometimes imperceptible, projections (Text-fig. 5, Figs. 5 to 12). The spores thus formed are in basifugal series, with the oldest spore at the base of the filament.

It was because of this particular method of spore development that Vuillemin (145) described and named the genus *Beauveria*. Since then it has been studied by various investigators (10, 34, 117, 122).

The filaments, when mature, are more or less comparable to a sympodium, which in the majority of cultures displays a scorpioid arrangement (Text-fig. 5, Fig. 16). The zigzag appearance is not always evident when the filament is covered with spores but becomes visible after the spores fall off.

Some of the less typical filaments display a helicoid arrangement (Text-fig. 5, Figs. 17, 19 to 22); others are straight with spores on one side (Text-fig. 5, Figs. 18, 27, and 30); occasionally the spore-bearing portion occurs directly on the mycelium (Text-fig. 5, Figs. 25, 26, 28, 31, and 33); and finally the entire sporogenous cell may have disappeared with the spores developing directly on the hyphal cells (Text-fig. 5, Fig. 29).

The spore-bearing filaments are extremely slender, varying from 0.5–1.0 μ in diameter. They also vary in length, according to the number of spores developed. The longest observed was 24 μ with 17 spores.

An interesting anomaly in sporogenous cell-formation is shown in Text-fig. 5, Fig. 32. The sporiferous stucture, instead of continuing to produce spores, grew vegetatively and gave rise to a normal-shaped sporogenous cell, which in turn produced a zigzag filament with spores. This phenomenon was also reported by Saccas (117) in his study of the species *B. heimii*.

More than one sporiferous filament has been reported on sporogenous cells from cultures of *B. globulifera* and *B. bassiana* (45, 95). This characteristic has been suggested as a means of differentiating some *Beauveria* species (45, 95, 108). In the present study, however, no spore-bearing element was observed that had developed more than one zigzag filament.

(*e*) *Arrangement of Spore Clusters*

It has been pointed out that, depending upon the growth conditions, vesicles and sporogenous cells among *Beauveria* isolates grow and multiply in mass, so that, in the later stages of development, they form compact, globose to oval clusters. Among these clusters there is some variation not only in size and shape but also in the arrangement or pattern of the groups.

In the more typical clusters, sporogenous cells are borne on vesicles that develop individually, in pairs, or in rows along one or both sides of the mycelium or conidiophores. The conidiophores may be of varying lengths, and also occur individually, in pairs, in rosettes, or occasionally in whorls along the mycelium. The clusters may be globose, 16–80 μ in diameter, or oval, 24–230 $\mu \times$ 20–40 μ. The less typical clusters result from the development of sporogenous cells in small groups directly on the conidiophores and/or mycelium.

Vuillemin (145) reported that the elements within the *Beauveria* clusters occur in whorls, and placed the genus in the group Verticilliae. In the present study it was concluded that the elements develop more frequently in clumps, groups, or clusters and that characteristic whorls are rarely formed. It is believed, therefore, to be more correct to treat this genus as a member of the group Botryidae of the order Moniliales, Fungi Imperfecti.

3. DISCUSSION

A study of numerous *Beauveria* isolates from 70 insect and four rodent species (see Observations II, A, 1, (*d*)) has shown that in general they vary only slightly as they develop on their natural hosts; they must in fact be transferred to artificial media before differences that exist among them are revealed. Siemaszko (122) apparently attached a great deal of importance to such morphological and cultural differences, for he recognized *B. globulifera* as a distinct species. He even considered that on Polish insects this species occurs in two forms, one giving, the other not giving, a wine-purple color to potato slabs, and moreover, that the forms in turn comprise a number of strains. This concept of strains was based in part on the fact that colonies of *B. globulifera* isolated from *Ips typographus* appeared slightly different from those isolated from *C. pomonella*, although both imparted color to potato slabs. He ultimately concluded that the appearance of a *Beauveria* form is essentially diagnostic of insect species having the same "general adaptation". Thus color-imparting *B. globulifera* is characteristic of those insect species that live permanently, or at least hibernate, under or in the bark of trees (bark beetles, *Carpocapsa* spp., etc.), whereas non-coloring *B. globulifera* is largely restricted to species that spend part of their life-cycle in the earth, on tree roots, or on fallen trees (*Diprion*, *Hylobius*, *Acanthocinus*, etc.).

Siemaszko further reported that the characteristics of a given strain are retained after passage through other insect species. He found that a *B. bassiana* strain from *Coccus coccus*, after passing through *I. typographus* and *Diprion pini*, retained in culture its original diagnostic attributes.

Herein, and for the reasons given below, most of the variation produced by isolates on different artificial media is not accorded specific status.

That no isolate is characteristic of a given insect species is shown by the occurrence of as much isolate-variability within as between insect species. This observation is in agreement with Steinhaus' (132) report that *Beauveria* isolated from *P. nubilalis* in some cultures resembed typical *B. bassiana* and in others tended towards *B. globulifera*. It has further been established herein that the original characteristics of various isolates undergo considerable modification when transferred to different types of media (see also Petch (95)), and in some cases when passed through other species of insects. Evidence that such spectacular transformations are not necessarily permanent has already been detailed.

Parenthetically, it is tempting to speculate that nutritional deficiencies might exist among some of the culture media or that physiological mutants

might exist among some of the isolates; work under way suggests that such cultures may perhaps be characterized by an inability to produce various essential vitamins and/or amino acids.

The evidence adduced above clearly demonstrates the close relationship existing among the *Beauveria* isolates studied. Their inconstant polymorphism suggests a grouping of all the entomogenous cultures studied under one or at the most two species, namely, *B. bassiana* and *B. tenella**. Their asexual mode of reproduction clearly precludes the adoption of genetic criteria, so fruitfully applied to sexual forms, so that, as is widely acknowledged, whether one or two species are recognized remains a matter of personal opinion. The aggregation of types reviewed earlier will accordingly be reconsidered in an evaluation of this concept.

B. Validity of Species Separation

It is evident from the present study that the original description of the genus *Beauveria* by Vuillemin (145) is not adequate to differentiate its members from those of other genera; its description has, therefore, been modified as follows:

Mycelium septate, branched, soft, procumbant to flocculent, mycelial blooms and/or coremia sometimes present; surface color variable, white to different shades of cream, yellow with admixtures of orange, red, and occasionally green; color in substatum variable, yellow to different shades of yellow-red, red, green and blue-green; conidiophores single or branched, oblong, cylindrical or flask-shaped, variable in length, bearing laterally, or at extremity vesicles giving rise to sporogenous cells; sporogenous cells generally globose, some cylindrical, flasklike, curved or straight; spores borne at threadlike apex of sporogenous cells on a series of branchlets arranged in a zigzag line more or less comparable to a cyme with scorpioid or helicoid dichotomy; conidia in basifugal series.

The sporogenous cells and vesicles develop and multiply in mass directly on the conidiophores and/or mycelium to form compact, globose to oval clusters or spore heads (Text-fig. 6).

The species pattern for the type species *B. bassiana* has also been broadened to include *Beauveria* species reduced to the status of strains or biotypes. Its new description follows:

Mycelial strands cylindrical 3.5 μ in diameter, hyaline and septate; conidiophores single or branched, oblong, cylindrical or flask-shaped, 2.5–5.5 μ × 1.5–2.5 μ to 15.5–25.5 μ × 1.5–3.0 μ, others form a sinuous mycelial type variable in length; vesicles globose 1.5–5 μ (average 2.5 μ), occasionally oblong; sporogenous cells generally globose, diameter variable

* *All cultures of B. bassiana and B. tenella were found to be heterogeneous in spore shape—the criterion that suggested their taxonomic separation. Those of the latter species were provided from old, much subcultured stocks; it is not improbable that their relative homogeneity (2% globose) may be a result of such protracted subculturing. Should fresh isolates from Melolontha spp. reveal heterogeneity of this character on a scale comparable with that of B. bassiana, the two species will perforce be merged.*

1.5–3.5 μ, others cylindrical 7.0–10.0 μ × 1.0–2.0 μ, curved and flask-like 7.5–15.0 μ × 1.0–3.0 μ, or thread-like filaments 7.5–28.0 μ or more in length; spores borne at thread-like apex of sporogenous cells on a series of zigzag branchlets, more or less comparable to a cyme with scorpioid or helicoid dichotomy; conidia in basifugal series; spore filaments 0.5–1.0 μ in diameter, variable in length; spores globose 1.0–4.0 μ in diameter to oval 1.5–5.5 μ × 1.0–3.0 μ (Text-fig. 6).

The sporogenous cells and vesicles develop and multiply in mass directly on the conidiophores and/or the main hyphal branches so that, in the final stage of development, they form compact globose to oval clusters.

The variation in size and shape of the conidiophores, vesicles, sporogenous cells, and spore clusters is influenced by the type of medium and the vitality of the fungus. These elements tend to disappear in some cultures following continued culturing.

The conclusions regarding *B. stephanoderis* are based on cultures received under this name from C.B.S. and A.T.C.C. Its structural morphology was found to be identical with that of *B. bassiana*. Such variations as occurred culturally were not sufficiently different from *B. bassiana* strains to justify its being treated as a separate species, the differences being largely the result of protracted subculturing on artificial media.

The globose vesicles supposedly characteristic of *B. laxa* are characteristic of *Beauveria* strains in general except that they occasionally vary in size and shape. The irregular shape of the spores is due to overcrowding. Generally the spores drop off readily, but not always, since numerous specimens have been examined in which sporogenous cells with their spore-clusters have been separated intact (Text-fig. 5, Fig. 16). Variability in the compactness of spore-clusters is also characteristic of numerous *Beauveria* cultures.

It will be readily appreciated that the structural and cultural variability of *B. bassiana* (see above) is of a magnitude sufficient to embrace *Botrytis necans*, *Beauveria stephanoderis*, and *Beauveria laxa*. Their reduction to synonymy with *B. bassiana* by the various authors mentioned is thus adequately confirmed.

The majority of isolates studied that were typically *B. globulifera* on one medium completely lost these characteristics and grew as typical *B. bassiana* when transferred to another medium. This held not only for widely different insect species but also for a series of isolates from an individual insect species. It soon became obvious that the amount of variability obtained was in large measure dependent upon the number of specimens examined within a species. Moreover, comparable variability resulted from single-spore cultures of so-called *globulifera*. Incidentally, these cultures showed a range of polymorphism identical with that of *B. bassiana*. It is apparent, therefore, that the characteristic upon which this species has been established is not valid as a criterion upon which to base a new species; "*B. globulifera*" should thus be treated as a strain of *B. bassiana*.

The demonstration that *Beauveria* isolates are capable of developing coremia validates the removal by Petch (95) of the species *vexans* from the genus *Isaria* as *Beauveria vexans*. It is evident from the illustrations given by Pettit ((100) Plate 3, Figs. 15-20) and from the present observations on cultural characteristics that this strain is in fact identical (as shown by the first-mentioned authors) with *globulifera* strains.

The conclusions regarding *B. effusa* in the present investigation are based on a study of cultures obtained from C.B.S. and A.T.C.C. The demonstration that in both structural and cultural characteristics this fungus is identical with *globulifera* is in agreement with Siemaszko.

Since *B. vexans*, *B. effusa*, *B. doryphorae*, and *B. delacroixii* are substantiated as forms of "*B. globulifera*", the merging of "*B. globulifera*" with *B. bassiana* reduces the foregoing species, with *globulifera*, to the status of strains of *B. bassiana*.

Beauveria tenella (= *B. densa*, see taxonomic review I, *C*) was not isolated from material received at this laboratory. Conclusions regarding it are therefore based on reports in the literature and on an examination of a few cultures obtained from other institutions. These cultures comprise one of "*B. densa*" from A.T.C.C. and three from C.B.S. identified as: *B. densa* (Link) Vuill. fr. *Melolontha* species; *B. densa* (Link) Vuill. (syn. *Botrytis tenella* Delacr.); and *B. densa* (Link) Vuill. strain LeMoult.

The *B. tenella* isolates resembled some of the more flocculent strains of *B. bassiana* that formed a wine-purple color on Sabouraud maltose agar. Their range of variation in gross morphology was not so wide as that shown by the *B. bassiana* strains, possibly owing to the limited number of isolates that could be obtained. Perhaps if sufficient numbers of cultures were isolated from numerous insects in widely diverse species, the range of variability might be comparable to that shown by *B. bassiana*.

Morphologically, *B. tenella* differs from *B. bassiana* only in spore size and shape (Plate I, Figs. 2 and 3; Text-fig. 6, Text-fig. 7). *Beauveria tenella* spores are mostly oval to ellipsoidal with a few globose; the oval spores vary from 2.0–6.0 μ × 1.0–4.0 μ in size, and the globose spores from 2.5–3.0 μ in diameter.

Among recently isolated *B. bassiana* cultures, 56.7% of the spores were globose, while among *B. tenella* cultures, 2% were globose*. Both species, therefore, are heterogeneous in spore shape, and may under certain conditions develop variation in size.

A fourth culture, obtained under the name of *Botrytis melolonthae* Sacc. from C.B.S., was derived from a culture originally isolated from diseased larvae of the cockchafer, *Melolontha vulgaris*, which were found in the Botanical Gardens at Pavino, Italy, in 1898. Petch (95) had concluded that

* *In a comparative study of two B. densa strains and five isolates representing the B. bassiana group, Benham and Miranda (11) conclude that B. densa constitutes a distinct species. They consider that it can be distinguished from the bassiana strains under investigation by its somewhat larger and more distinctly elongated spores, the long and slender "conidiophores" (sporogenous cells), and by an apparent vitamin deficiency.*

the size and shape of the spores (oval, 2.5–3 μ × 1.5 μ) were similar to those of the fungus *Botrytis tenella* Delacr., i.e. *Sporotrichum densum* Link. At the same time, however, he stated that it was not evident from Saccardo's description (116) that it belonged to the genus *Beauveria*.

Here this fungus is found, on morphological and cultural grounds, to be a member of *Beauveria; Botrytis melolonthae* is accordingly synonymous with *Beauveria tenella* (Delacr.) Siem.

Published descriptions of *Beauveria brongniartii* confirm, in the writer's opinion, Siemaszko's classification of this fungus as a strain of *B. tenella*.

No material of *Beauveria peteloti* has been examined. A review of Vincens' original publication (142) and observations by Petch (97) exclude it from *Beauveria*. Two of the sporogenous cells in Vincens' illustrations ((142), Plate iv, Figs. *m*, *o*) resemble to some degree those of the genus *Beauveria*. The zigzag filamentous structures that Vincens observed on the thorax of *P. canadensis* and the *Apis* sp. ((142), Plate iv, Fig. r; v, Figs. v, x, respectively) resemble in a general way atypical forms lacking sporogenous cells (see Text-fig. 5, figs. 25, 26, 28, 31 herein), such as are occasionally found amoung *Beauveria* cultures. At the same time, however, as suggested by Petch (97), the zigzag spore-bearing filaments are more characteristic of normal conidiferous hyphae of a *Sporotrichum*, undifferentiated in diameter from the hyphae, of which they are continuations. It is thus apparent that a new *Beauveria* species is not involved. The writer favors the conclusion drawn by Petch (97). According to him the three insect species were attacked by a fungus belonging to the genus *Hirsutella* and close to *H. saussurei*; this fungus in turn had been attacked by a *Sporotrichum* sp. The three collections illustrate progressive stages of mouldiness.

Vincens apparently considered the zigzag spore-bearing filaments of the species *B. oryzae* as a characteristic placing it in *Beauveria*. It is evident, however, that the stiffly upright conidiophores bearing sporogenous cells, as shown in Vincens' illustrations ((143), Fig. 2, p. 122), are more characteristic of *Tritirachium*. This species should, therefore, be assigned to the newer form genus *Tritirachium*. It is of interest to note that this species closely resembles *T. roseum*, and *T. purpureum* in both color and morphological characteristics.

No material of *Beauveria paranense* has been seen. The conclusions reached here, however, are the same as those of Marchionatto (76). It is apparent from his description and illustrations (Fig. 1*a*, *b*, *c*) that it is not a *Beauveria* species and should therefore be designated *Sporotrichum paranense* (March.).

Material of *Botrytis rileyi* has not been examined, but its original description excludes it from the genus *Beauveria*.

Material of *Beauveria coccorum* has not been examined. It is evident, however, from the morphology of *Gonatorrhodiella coccorum* as illustrated by Petch ((93), Plate X, Figs. 18, 19, 20, 21) that this fungus does not resemble members of *Beauveria*, in which it was placed by Linder (69). It is suggested that, because of the uncertainty of Petch's descriptions, the original name of

the fungus be retained as *Rhinotrichum album* until it is encountered again and properly described. Similarly, and because of its close resemblance to *R. album* (96), it is considered that *B. coccospora* should remain as *R. depauperatum* pending reisolation and further description.

The following account of *Beauveria heimii* is based on Saccas' description (117) and on a culture received from France: Colony dense, flaky, circular mat 0.5 cm. in diameter, 1 mm. in height, clear beige in color; mycelium irregular in outline, branched, septate, subhyaline to light yellowish, 3 μ in diameter; conidiophores brittle, fragile, erect, length variable, 450 μ–1.3 mm., diameter 3 μ, brownish-yellow; at a certain height (usually about one quarter of its normal length) the conidiophores develop a whorl of branches (usually three or four, sometimes two, rarely one) at an angle of 35°–70° from the axis (Text-fig. 8, Fig. 1); a second whorl is formed 30–50 μ above the first, (then successive whorls arise during development) until at maturity it may have from 4 to 10 verticils (Text-fig. 8, Fig. 1); the primary verticil may in turn branch into a secondary series and sometimes a tertiary series of three or four branches (Text-fig. 8, Fig. 1); branches in a series vary from 20 to 30 μ in length and 3 to 4 μ in diameter; the conidiophores become progressively thinner near the summit, where the last and often the second last whorls are composed entirely of sporogenous cells (Text-fig. 9, Fig. 1). Sporogenous cells occur laterally at the extremity of each of the outer branches in whorls of three to six (usually four), and at the apex of the conidiophores (Text-fig. 8, Figs. 1, 2, 3; Text-fig. 9, Figs. 1, 3, 4), yellowish-brown, varying in shape from bottle-like, 8.0–13.5 μ in length and 2.5–3.5 μ in diameter at their widest part, basal portion *ca.* 1.5 μ in diameter (Text-fig. 8, Fig. 4), to spindle- or hypha-like 13.0–27.0 μ in length and 1.5–2.5 μ in diameter (Text-fig. 9, Figs. 2, 6, 7) tapering towards the tip to form zigzag thread-like filaments varying from 15.0 to 24.0 μ in length and 1.0 to 15.0 μ in diameter. Spores develop alternately in a basifugal manner at the angle of each broken link, yellowish-brown, oval, sometimes pyriform, or globose, 2.0–3.5 μ in diameter, or 2.5–5.0 $\mu \times$ 2.0–4.0 μ in size. The basilar portion of the spore is flat and to it is attached a small disk that is scarcely visible (Text-fig. 9, Fig. 5). The disk acts as a juncture between the spore and the broken axis of the sporogenous cell.

Saccas concluded that, since this fungus forms spores in a manner similar to that of members of the genus *Beauveria*, it should be so treated. It is apparent, however, from a comparison of the illustrations of *heimii* with that of *B. bassiana*, that the former is not a *Beauveria* species and should rightly be assigned to *Tritirachium*.

It should be noted that *T. heimii* resembles *B. dependens* if not so closely as to suggest identity at least sufficiently so to warrant reinstating the latter also in the genus *Tritirachium*. By extension, the same reasoning calls for the reassignment of *B. alba* and *B. spicata* to *Tritirachium*.

As briefly mentioned in Taxonomic review, II, *Tritirachium roseum*, *T. musae*, *T. purpureum*, and *T. cinnamomeum* strictly conform to the generic

requirements as established by Limber. Similarly, Langeron's transfer of his own species, *Beauveria brumpti*, to this genus seems adequately justified by Brumpt's ((22) Fig. 967) illustration that its conidiophores are stiffly upright with sporogenous cells occurring in whorls directly along the main axis. The conidiophores in Brumpt's figure are, moreover, apparently simple, and in this way resemble those of *T. album*.

No material of *Tritirachium epigaeum* has been examined. Two sporogenous cells, however, were noted in Aschieri's (1) Fig. 3 *b* (to have three and five spores, respectively) that taper toward their apices and appear to form a zigzag sporebearing filament characteristic of both *Beauveria* and *Tritirachium*. These are the only illustrations in this paper that justify the inclusion of *epigaeum* in one or other of these two genera.

Tritirachium epigaeum differs from typical *Beauveria* species in the absence of vesicles and from typical *Tritirachium* species in the absence of stiffly erect conidiophores. Apart from these differences, the general size and shape of the conidiophores and sporogenous cells of *T. epigaeum* are not unlike some of those found in *Beauveria*. A final generic disposition for this species must, therefore, remain in abeyance.

Kuru's (62) description and illustrations of "*Isaria shiotae*", contrary to those of Langeron (65), suggest that, this species is a *Beauveria* and, in fact, closely related (because of its spore size and shape) to the species *B. tenella* (Delacr.) Siem.

No material of *Tritirachium rubrum* has been examined. The occurrence of spores in chains, however, is more characteristic of *Spicaria* than of either *Beauveria* or *Tritirachium*. It thus appears that Dodge's (36) classification is probably the correct one, and that this species should be placed in the genus *Spicaria* as *S. rubra*.

From the descriptions and illustrations by Froilano de Mello ((47), 5*b*, Figs. 7, 8, 9, 10), the writer considers *Tritirachium viannai* to belong to neither this genus nor to *Beauveria*.

C. Discussion

A thorough review of the literature shows that 32 named species have been involved in the genera *Beauveria* and *Tritirachium*. The descriptions of 14 of these are characteristic of *Beauveria*, one is regarded as uncertain as to genus, 10 belong to *Tritirachium**, and the remaining seven belong to neither.

From the general investigation on the morphological and cultural characteristics used in the identification of *Beauveria* species, it was concluded that *B. bassiana* and *B. tenella* are the only two members that warrant species status. Another 12 so-called *Beauveria* species, since their descriptions were not based on diagnostic characteristics, are simply strains of either *B. bassiana* or *B. tenella*. *Beauveria stephanoderis*, *B. laxa*, *B. globulifera*, *B. effusa*,

* *Another species Tritirachium hydnicola has been mentioned in a recent publication by Hughes. (Hughes, S. J. Conidiophores, conidia, and classification. Can. J. Botany, 31: 577-660. 1953.)*

B. vexans, *B. doryphorae*, *B. delacroixii*, and *B. acridiorum* are strains of *B. bassiana*, while *B. densa*, *B. melolonthae*, *B. brongniartii*, and *B. shiotae* are strains of *B. tenella*.

The species *T. dependens*, *T. album*, *T. spicatum*, *T. oryzae*, *T. heimii*, *T. brumpti*, *T. musae*, *T. purpureum*, *T. cinnamomeum*, and *T. roseum* are characteristic of the form genus *Tritirachium*. "*Tritirachium epigaeum*" is the dubious species.

The *Tritirachium* species resemble members of the genus *Beauveria* in that they produce sporogenous cells that end in a tapered zigzag filament, like a rachis of a spike of wheat, and carry spores alternately on the angles (13, 14, 65, 117, 168). Both genera are cosmopolitan. An examination of members of both genera, however, has shown that there may be some fundamental differences between the sporiferous elements produced in both. For example, in *Beauveria*, the filament is characteristically a zigzag consisting of a series of short sharp turns or angles, whereas in *Tritirachium* the angles are much less abrupt and seem to be more or less sinuous in appearance. This may be due to the fact that in *Beauveria* the spores are borne on short slender stalks, whereas in *Tritirachium* the spores appear more or less sessile with a flat, disk-shaped basilar element acting as a juncture between the broken axis of the sporogenous cell and the spore. Moreover, in *Tritirachium* the filaments are usually much longer. Some have been observed over 110 μ in length and 0.5–1.5 μ in diameter, whereas in *Beauveria* none has been observed much over 24 μ in length and 0.5–1.0 μ in diameter.

In *Tritirachium* the conidiophores are erect, cylindrical, hypha-like structures varying in length from 400 to 1300 μ. They occur individually, directly on the main hyphae of the colony, and become progressively thinner near the apex. The conidiophores give rise to a whorl of branches (3–4), usually about one-fourth of its normal length, a second whorl is formed 30–50 μ above the first, then successive whorls develop during growth, until at maturity there may be from 4 to 10 verticils. The primary verticil may in turn branch into a secondary series and sometimes a tertiary series of three or four branches. Development of the branches diminishes progressively from the base to the tip of the conidiophore, where the last, and often the next to last, whorls are composed entirely of sporogenous cells. It is in this way that the shrub-like appearance of the colony is formed. In *Beauveria* the conidiophores are generally in the form of undifferentiated hyphae that exist as single or branched lateral stalks (variable in size) along the axis of the main hyphae. They may be globose or oblong or cylindrical ranging from 2.5 to 28.0 $\mu \times$ 1.5 to 2.5 μ in size. Occasionally an extremely long sinuous mycelial type of conidiophore is formed. The conidiophores may occur individually, in twos, or in thick rosettes, but seldom in whorls along the main hyphae of the fungus colony.

In *Tritirachium* there are no vesicles, the sporogenous cells developing in whorls on the outer edge of the primary, secondary, and tertiary branches, and at the tips of the conidiophores. In some species the conidiophores

TEXT-FIG. 1. Variation in size and shape of spores among isolates of *B. bassiana* strains.

Secondary and normal spores borne together on hypha-like sporogenous cells.
FIGS. 1, 5. *bassiana* on *N. tsugae*.

Secondary spores borne directly on the mycelium.
FIGS. 2, 3. *bassiana* on *Tenthredinidae*.
FIG. 4. *bassiana* on *N. tsugae*.
FIG. 10. *globulifera* on *S. rufimitrana*.
FIGS. 11, 12. *globulifera* on *N. canosaria*.

Flask-shaped sporogenous cell with a cluster of secondary spores.
FIG. 6. *bassiana* on *N. tsugae*.

Secondary spore borne on a hypha-like sporogenous cell.
FIG. 7. *bassiana* on *N. tsugae*.

Flask-shaped sporogenous cells with normal and secondary spores.
FIG. 8. *bassiana* on *N. tsugae*.
FIG. 9. *bassiana* on *N. abietis*.

Variation in size and shape of spores from 6 months old cultures.
FIG. 13. *globulifera* on an unknown insect species, B.C., Canada.
FIG. 14. *globulifera* on *P. erichsonii*.
FIG. 15. *bassiana* on *Phalaenidae*.

TEXT-FIG. 2. Spore germination in strains of *Beauveria* species.

Spores with 1 to 3 germ tubes that developed at various places on the spore.
FIG. 1. *bassiana* on an unknown insect sp., Dept. of Agr., Ottawa.
FIGS. 2, 13. *tenella*, "*B. densa* (Link) Vuill. fr. *Melolontha* spp." from C.B.S.

Variation in length of germ tubes at the end of 8 hours.
FIG. 6. *stephanoderis* received from C.B.S.
FIG. 9. *tenella*, "*B. densa* (Link) Vuill. *Melolontha* spp." from C.B.S.
FIG. 10. *bassiana* on an unknown insect sp., Dept. Agr., Ottawa.

Variation in length of germ tubes at end of 16 hr.
FIGS. 4, 7. *globulifera* on *C. pomonella*.
FIG. 8. *effusa* received from C.B.S.

Germ tubes with cross walls.
FIG. 3. *effusa* received from C.B.S.
FIG. 5. *stephanoderis* received from C.B.S.
FIG. 13. *tenella*, "*B. densa* (Link) Vuill. *Melolontha* spp." from C.B.S.

Germ tube, showing initial state in development of a secondary branch.
FIG. 12. *globulifera* on *C. pomonella*.

Germ tube with secondary branches at the end of a 24 hr. period.
FIG. 11. *effusa* received from C.B.S.

Germ tube, showing initial stage in development of a tertiary branch.
FIG. 14. *stephanoderis* received from C.B.S.

TEXT-FIG. 3. Variation in size and shape of conidiophore among isolates of different strains of *Beauveria* species.

A fungus cluster borne directly on the mycelium.
FIG. 1. *bassiana* on *P. erichsonii*.

An oblong conidiophore with sporogenous cells.
FIG. 2. *globulifera on Choristoneura pini*.

Flask-shaped conidiophores with sporogenous cells.
FIG. 4. *globulifera* on *S. rufimitrana*.
FIG. 5. *globulifera* on *N. swainei*.
FIG. 6. *bassiana* on *N. banksianae*.
FIG. 7. *bassiana* on *S. duplex*.
FIG. 8. *globulifera* on *N. canosaria*.

Typical unbranched cylindrical conidiophores with sporogenous cells.
FIG. 3. *globulifera* on *M. americanum*.
FIG. 12. *effusa* from C.B.S.
FIG. 13. *bassiana* on *P. nubilalis*.
a. vegetative hyphae *d*. basal portion of sporogenous cells.
b. conidiophores *e*. terminal portion of sporogenous cells.
c. vesicles *f*. spores.

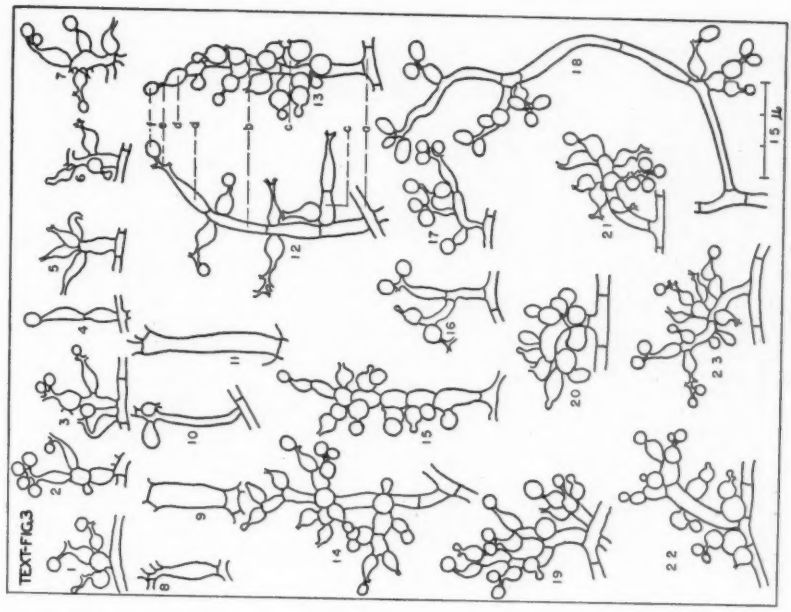
Atypical unbranched cylindrical conidiophores with sporogenous cells.
FIG. 9. *bassiana* on *P. nubilalis*.
FIG. 10. *bassiana* on an unknown insect species, Ottawa.
FIG. 15. *bassiana* on *P. nubilalis*.
FIG. 17. *globulifera* on *N. swainei*.
FIG. 19. *globulifera* on *H. textor*.
FIG. 20. *bassiana* on *N. banksianae*.
FIG. 21. *bassiana* on *C. pomonella*.
FIG. 22. *globulifera* on *E. chalcedona*.
FIG. 23. *globulifera* on *N. lecontei*.

A conidiophore section.
FIG. 11. *heimii*, isolated from paper (See *T. heimii*.)

A short cylindrical conidiophore with a secondary branch and sporogenous cells.
FIG. 16. *bassiana* on *M. juniperinus*.

A variously branched conidiophore with sporogenous cells.
FIG. 14. *globulifera* on *E. chalcedona*.

A sinuous type of conidiophore.
FIG. 18. *tenella*, obtained as *Botrytis melolonthae* from C.B.S.



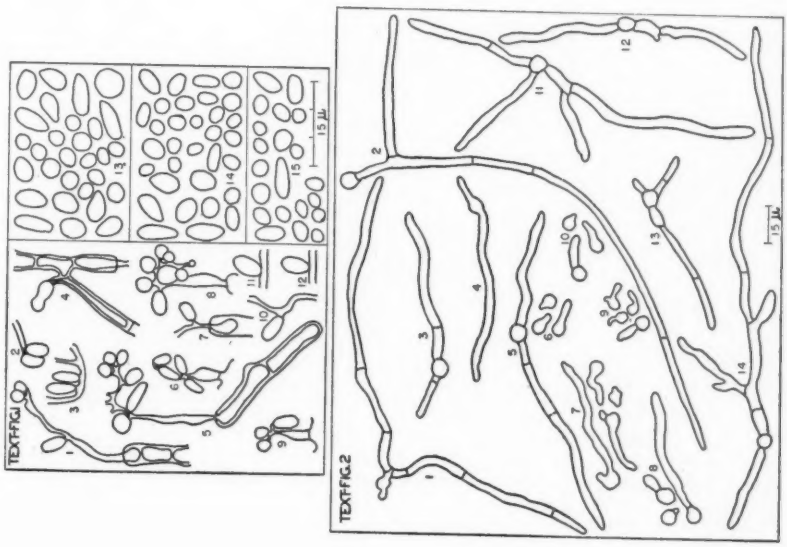


FIG. 14. *stephanoderis* received from C.B.S.

TEXT-FIG. 4. Variation in size and shape of the basal section of sporogenous cells among strains of *Beauveria* species.

Globose to oval sporogenous cells.
FIG. 1. *tenella* as *Botrytis melolonthae* from C.B.S.
FIGS. 2, 3. *stephanoderis* from C.B.S.
FIG. 4. *globulifera* on *E. chalcedona*.
FIGS. 5, 6. *stephanoderis* from A.T.C.C., Washington.
FIG. 7. *globulifera* on *A. cerasivorana*.
FIG. 8. *bassiana* on *B. mori*.

A globose vesicle with a cluster of oblong sporogenous cells.
FIG. 9. *bassiana* on *M. juniperinus*.

Globose vesicles with globose to oblong to flask-like sporogenous cells.
FIGS. 10, 11. *globulifera* on *M. vaccinii*.

A conidiophore with globose and cylindrical shaped sporogenous cells.
FIG. 12. *bassiana* on *M. juniperinus*.

Cylindrical-shaped sporogenous cells.
FIG. 13. *globulifera* on *N. antiopa*.
FIG. 14. *globulifera* on *P. erichsonii*.

Flask-shaped sporogenous cells.
FIG. 15. *globulifera* on *Choristoneura pini*.
FIG. 16. *heimii* on paper. (See *T. heimii*.)
FIG. 19. *globulifera* on *N. canosaria*.

Curved, flask-shaped sporogenous cells.
FIGS. 17, 18. *globulifera* on *P. acerifoliella*.
FIG. 20. *globulifera* on *N. canosaria*.

Hypha-like sporogenous cells.
FIGS. 28, 32. *globulifera* on *M. disstria*.

Hypha-like curved sporogenous cells.
FIG. 21. *globulifera* on *T. triangulum*.
FIG. 23. *globulifera* on *C. fumiferana*.
FIG. 24. *globulifera* on *N. lecontei*.
FIG. 25. *globulifera* on *P. erichsonii*.
FIG. 26. *tenella* from A.T.C.C.
FIGS. 27, 29. *globulifera* on unknown insect sp., B.C.

Variation among sporogenous cells occurring in same cluster, borne directly on mycelium.
FIG. 22. *globulifera* on *T. triangulum*.
FIG. 30. *globulifera* on *C. fumiferana*.
FIG. 31. *globulifera* on *P. erichsonii*.
FIG. 33. *globulifera* on *N. antiopa*.

TEXT-FIG. 5. Variation in terminal section of sporogenous cells among strains of *Beauveria* species.

Method of spore formation among *Beauveria* species.
FIGS. 1 to 12, inclusive. *tenella* obtained from C.B.S. as "*B. densa* Link (Vuill.) on *Melolontha* sp."
a. spore No. 1, *b*. spore No. 2, *c*. spore No. 3. N.B. Spores form in a series of branchlets on a zigzag line.

Sporogenous cells in which zigzag terminal filament is almost completely covered by small compact cluster of spores.
FIG. 13. *stephanoderis* from C.B.S.
FIG. 14. *globulifera* on *Choristoneura pini*.
FIG. 15. *globulifera* on a Geometrid.

A typical sporogenous cell with terminal section showing scorpioid dichotomy.
FIG. 16. *bassiana* on *P. japonica* (2980 ×).

Sporogenous cells with terminal section showing helicoid dichotomy.
FIG. 17. *globulifera* on *C. pellucida*.
FIG. 19. *globulifera* on *Choristoneura pini*.
FIG. 20. *bassiana* on *H. crocea*.
FIGS. 21, 22, 24. *B. bassiana* on *N. tsugae*.

Spores borne on one side of a straight or erect terminal filament.
FIG. 18. *globulifera* on *E. americana*.
FIGS. 27, 30. *bassiana* on *N. tsugae*.

Sporiferous filaments borne directly on mycelium.
FIGS. 25, 26. *globulifera* on *P. erichsonii*.
FIG. 28. *tenella* received from C.B.S. as "*B. densa* Link (Vuill.) LeMoult."
FIG. 31. *globulifera* on *S. rufimitrana*.
FIG. 33. *tenella* received from C.B.S., as "*B. densa* (Link) Vuill. on *Melolontha*."

A single spore borne on a hypha-like projection.
FIG. 23. *bassiana* on *N. tsugae*.

A single spore borne directly on a hyphal cell.
FIG. 29. *bassiana* on *N. tsugue*.

Sporogenous cell with a sporiferous filament that continued to develop vegetatively and produced another sporogenous cell with a fertile filament.
FIG. 32. *globulifera* on *E. americana*.

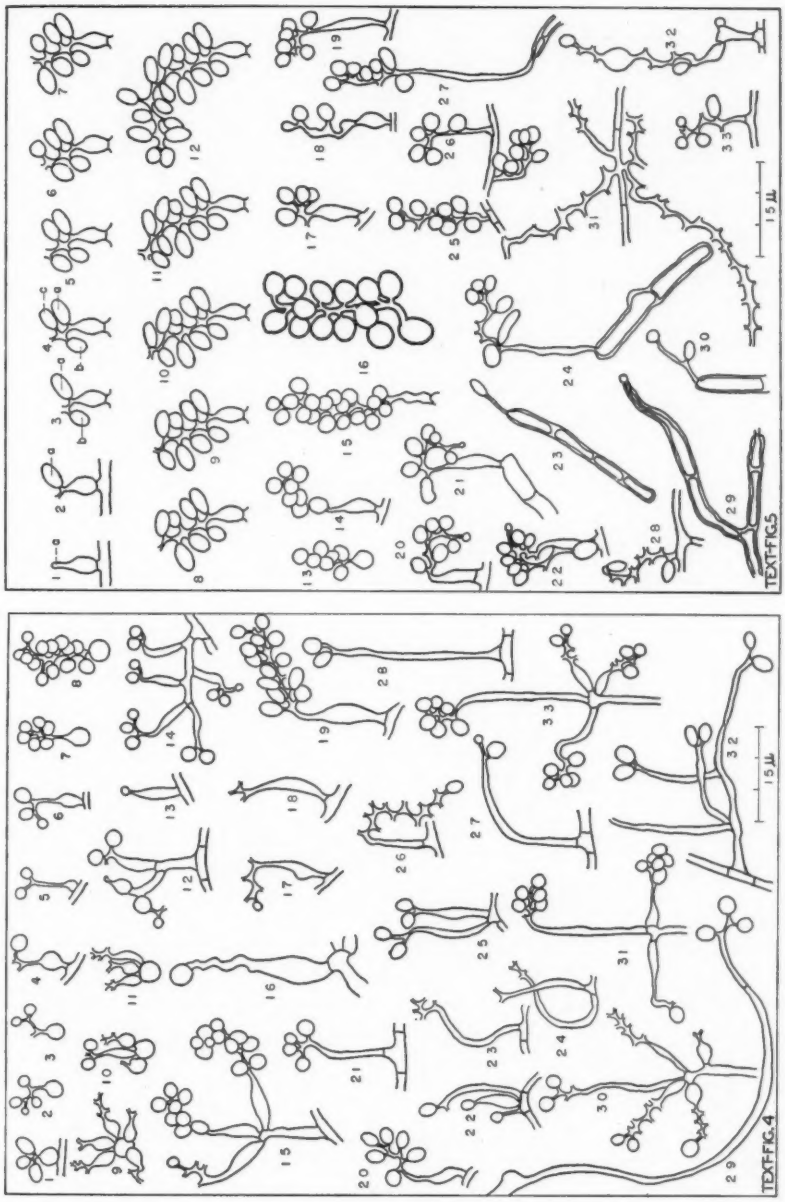


FIG. 33. *globulifera* on *N. antiopa*.

TEXT-FIG. 6. Structural elements of *B. bassiana* strains isolated from different insect species.

A. From *Lambdina sommiaria*
FIGS. 1, 2, 3. Vesicles with variously shaped sporogenous cells.
FIG. 4. Mycelial section with conidiophores and variously shaped sporogenous cells.
FIG. 5. Atypical sporogenous cell.

B. From *Oporinia autumnata*
FIGS. 1, 2, 3. Mycelial sections with variously shaped sporogenous cells.
FIG. 4. A vesicle with sporogenous cells.
FIGS. 5, 6, 7, 8. Sporogenous cells.

C. From *Popillia japonica*
FIG. 1. Sporogenous cells occurring directly on mycelium.
FIGS. 2, 3, 4, 5. Mycelial sections with conidiophores and sporogenous cells.

D. From *Crambus* sp.
FIG. 1. Sporogenous cells occurring directly on mycelium.
FIGS. 2, 3, 5. Mycelial sections with vesicles and sporogenous cells.
FIG. 4. Mycelial section with conidiophore, vesicles, and sporogenous cells.
FIG. 6. An atypical sporogenous cell.

E. From *Neodiprion tsugae*
FIGS. 1, 3, 5. Mycelial sections with conidiophores and sporogenous cells.
FIG. 2. Sporogenous cell occuring directly on mycelium.
FIG. 4. Vesicles and sporogenous cells.

F. From *Venusia cambrica*
FIG. 1. Mycelial section with conidiophore and sporogenous cells.
FIGS. 2, 4. Mycelial sections with vesicles and sporogenous cells.
FIGS. 3, 5. Vesicles and sporogenous cells.
FIG. 6. Mycelial section with a conidiophore, vesicles and sporogenous cells.

G. From *Sitona cylindricollis*
FIG. 1. Mycelial section with vesicles and sporogenous cells.
FIGS. 2, 4. Mycelial section with conidiophores, vesicles, and sporogenous cells.
FIG. 3. Mycelial section with sporogenous cells.

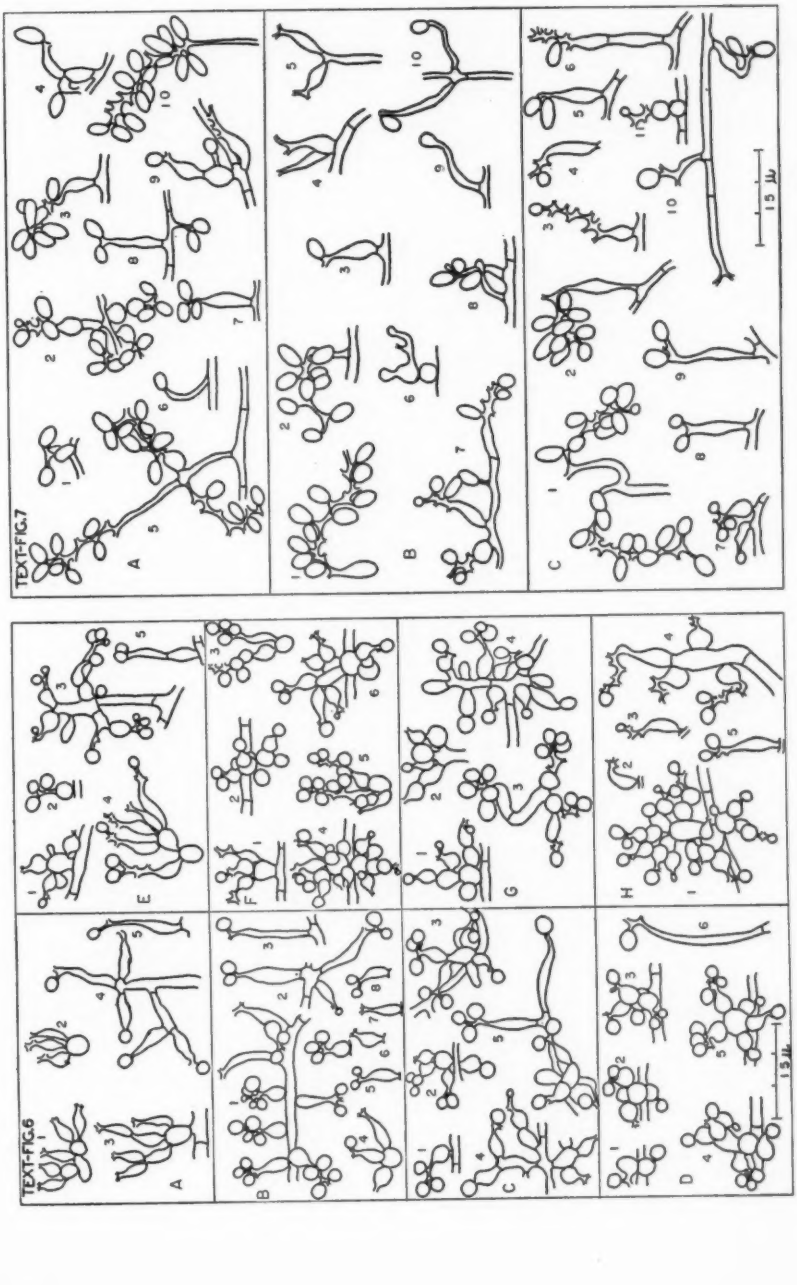
H. From *Pyrausta nubilalis*
FIG. 1. Mycelial section with conidiophore, vesicles, and sporogenous cells.
FIGS. 2, 3, 5. Sporogenous cells.
FIG. 4. Sporogenous cells occurring directly on mycelium.

TEXT-FIG. 7. Structural elements of the species *B. tenella*.

A. A culture of *B. tenella* obtained from C.B.S. as "*B. densa* (Link) Vuill. fr. *Melolontha* sp."
FIG. 1. A zigzag spore bearing filament borne directly on a mycelial section.
FIG. 2. A mycelial section with a conidiophore, vesicle, and sporogenous cells.
FIGS. 3, 6, 7, 8. Mycelial sections with sporogenous cells borne directly on the mycelium.
FIGS. 4, 5, 9. Mycelial sections with conidiophores and sporogenous cells.
FIG. 10. Hypha-like sporogenous cell with spores.

B. A culture of *B. tenella* obtained from C.B.S. as "*B. densa* (Link) Vuill. (Syn. *Botrytis tenella* Delacr.)."
FIG. 1. A sporogenous cell with spores.
FIGS. 2, 3, 4, 5, 7, 8, 9, 10. Mycelial sections with sporogenous cells borne directly on the mycelium.
FIG. 6. A mycelial section with a vesicle and sporogenous cell. The irregularity in the size and shape of the sporogenous cells is due to protracted subculturing on artificial media.

C. A culture of *B. tenella* obtained from C.B.S. as "*B. densa* (Link) Vuill. strain Le Moult."
FIGS. 1, 2, 3, 5, 7, 8, 9, 10. Mycelial sections with variously shaped sporogenous cells borne directly on mycelium.
FIG. 4. A sporogenous cell.
FIG. 6. A mycelial section with a conidiophore and sporogenous cell.
FIG. 11. A mycelial section with a vesicle and sporogenous cell.



genous cells.
FIGS. 2, 3, 5. Sporogenous cells.
FIG. 4. Sporogenous cells occurring directly on mycelium.

TEXT-FIG. 8, TEXT-FIG. 9. Structural elements of *T. heimii*.
This species, isolated from paper, was obtained from the National Museum of Natural History in Paris, France.

TEXT-FIG. 8.

FIG. 1. Stiffly upright conidiophores, verticillately branched, usually with three branches in each whorl. The branches of the primary verticil may in turn give rise to a secondary series and these in turn to a tertiary series of three or four branches.

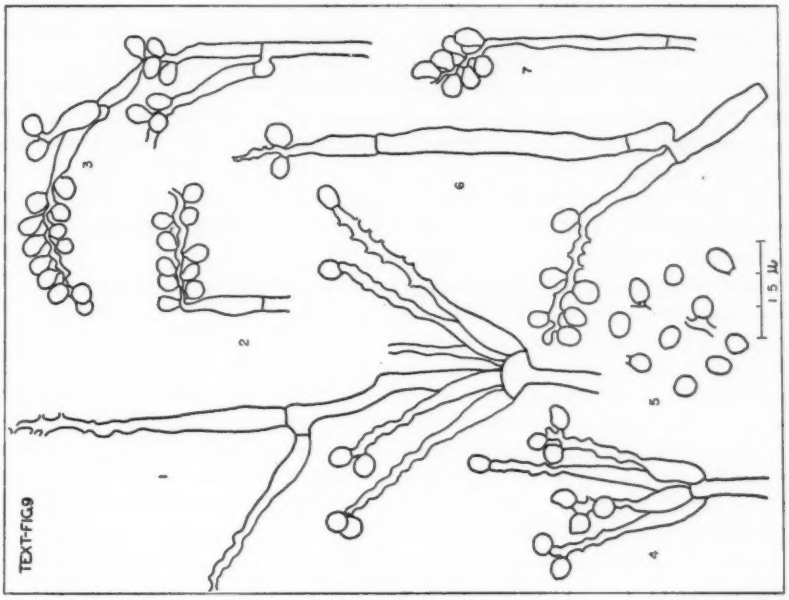
The sporogenous cells are borne laterally at the extremity of each of the outer branches in whorls of 3-6, or directly at the apex of the conidiophores.

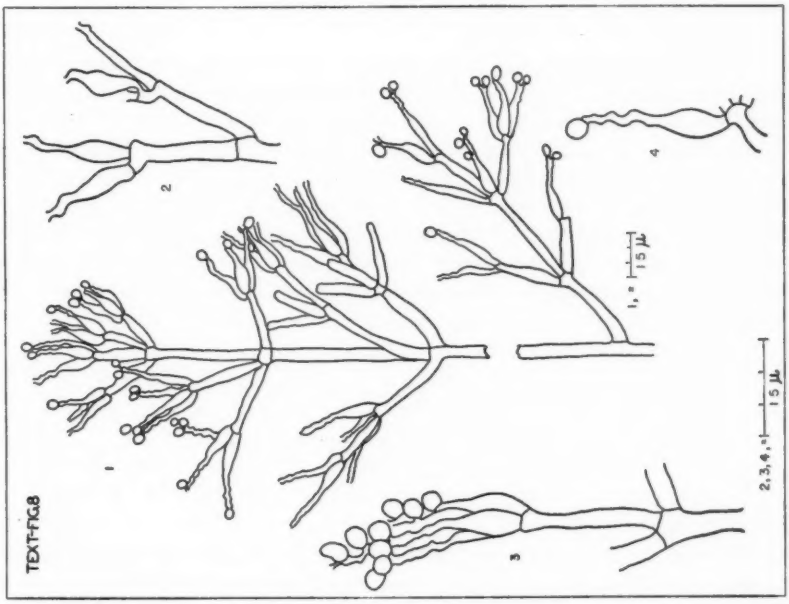
Complex conidiophores of a similar nature are also characteristic of *T. dependens* and *cinnamomeum*.

FIGS. 2, 3. Segments of conidiophores with sporogenous cells (see also Text-fig. 3, Fig. 11).

FIG. 4. A typical flask-shaped sporogenous cell terminating in a zigzag spore bearing filament (see also Text-fig. 4, Fig. 16).

TEXT-FIG. 9. FIG. 1. The upper portion of a conidiophore, which becomes progressively thinner near the summit, where the last and often the second last whorls are composed entirely of sporogenous cells. FIG. 2. A sporogenous cell with the zigzag spore bearing filament occurring at right angles to the main axis. FIGS. 3, 6. Segments of conidiophores with sporogenous cells terminating in zigzag spore bearing filaments. FIG. 4. A portion of an outer branch bearing a cluster of variously shaped sporogenous cells. FIG. 5. Spores, pyriform to globose to oval in shape. Note that they develop on a flat disk-like element. FIG. 7. A hypha-like sporogenous cell.


TEXT-FIG 9
1
2
3
4
5
6
7
15 μ


TEXT-FIG 8
1
2
3
4
1, 2 15 μ
2, 3, 4, 15 μ

appear to be simple, and the sporogenous cells in such cases develop directly on the conidiophores. In *Beauveria* the sporogenous cells may develop directly on the mycelium and/or the conidiophores, but as a general rule they occur on globose to oblong intermediary cells or vesicles. These give rise at maturity to sporogenous cells, secondary vesicles, or both secondary vesicles and sporogenous cells. The secondary vesicles in turn give rise to another series of vesicles and sporogenous cells. This process is repeated several times until, in the final stages of development, there exists a more or less rounded, compact cluster of vesicles, sporogenous cells, and spores (16–80 μ in diameter, or 24–230 $\mu \times$ 20–40 μ in size).

In *Tritirachium* the sporogenous cells are variable, flask-shaped to hypha-like, ranging from 6.5 to 31.0 μ in length and 1.5 to 2.5 μ in diameter, and disposed in whorls. In *Beauveria* the more typical sporogenous cells are globose, 1.5–3.5 μ in diameter. These occasionally become irregular in shape owing to crowding. The atypical sporogenous cells are quite variable in shape and size, cylindrical, flask-shaped, straight, or curved thread-like, 7.0–28 $\mu \times$ 1.0–3.5 μ. A few may be extremely long, forming a sinuous mycelial type of spore-bearing filament. The sporogenous cells may occur individually, in twos, or in clusters, directly on the mycelium, on the conidiophores, or on the vesicles.

In *Tritirachium* the spores vary from globose, 1.5–3.0 μ in diameter, to pyriform, oval, or ellipsoidal, 1.5–5.0 $\mu \times$ 1.5–4.0 μ in size; in *Beauveria* the spores vary from globose, 1.0–4.0 μ in diameter, to oval to ellipsoidal, 1.5–6.0 $\mu \times$ 0.5–4.0 μ in size.

Cultures of some *Tritirachium* species (13, 14) develop very limited growth which is bushlike in appearance, whereas the *Beauveria* species spread out rapidly on artificial media to form a flat or an elevated mycelial growth.

From the evidence collected in this investigation, it is concluded that members of the *Tritirachium* group can be readily distinguished morphologically from *Beauveria* species. *Tritirachium* species should not, therefore, be transferred to the genus *Beauveria* as suggested by Saccas (117).

The species *B. peteloti*, *B. rileyi*, *B. paranense*, *B. coccorum*, *B. coccospora*, *T. rubrum*, and *T. viannai* are not characteristic of either *Beauveria* or *Tritirachium* and have been placed in other genera.

II. VIRULENCE

A. Members of the Genus *Beauveria*

1. on insects

(*a*) *Historical*

The term virulence as defined by Dubos (39) does not refer to one specific attribute of the infective microorganism, but rather expresses the ability of a given agent to establish, under well-defined conditions, a pathological state

in a given host. According to him, the infectious agent must be able to penetrate the protective barriers that shelter the host from the environment; it must be capable of surviving the many defense mechanisms, cellular and humoral, that attack it as soon as it reaches the tissues; it must find an environment favorable for multiplication; and, finally, it must be able to produce disease, i.e. substances or conditions that cause physiological and pathological disturbances.

In general, the various components included under virulence have not been thoroughly investigated in the study of the host–parasite relationships among members of the genus *Beauveria*. For example, there is no information in the literature regarding the stages before penetration (spore germination; early growth and behavior of germ tubes; influence of stimuli, etc.).

The fact that there may be certain mechanical barriers to overcome has been suggested by Sussman (133). He found that the pupae of *Platysamia cecropia* L. were more resistant to infection by *Aspergillus flavus* Link than were the larvae, and attributed this to the presence of a lipid protective layer on the epicuticle that presents an effective barrier to infection from the outside.

With regard to *Beauveria* species, it has been pointed out that, among such insects as the sugar-beet beetle (88) and the cockchafer (56), all stages are susceptible to infection. Among such others as the Philippine migratory locust (109) and the corn borer (7), the earlier instars are more susceptible than the advanced stages. In the Colorado potato beetle (91), the larvae and adults are the most susceptible, the pupae much less so, and the eggs are completely resistant to attack.

Investigations on the process of infection by *B. bassiana* of larvae of *B. mori*, *P. nubilalis*, and *Pieris brassicae* (Linn.) have been conducted by Paillot (90), Lefebvre (67), and Pilat (103), respectively. This work has been summarized to some extent by Sussman (134) in his recent histopathological study of *A. flavus* on *P. cecropia*. Sussman has reported that the pathogen in most cases enters the animal through the gastrointestinal tract, although it is possible for infection to occur by way of the integument through the intersegmental membranes. Upon gaining entrance, the fungus attacks the fat body of the insect and spreads extensively throughout this tissue, even before it begins to attack the other tissues. Complete consumption of the fat body is achieved by the fungus, whereupon the area formerly occupied by this tissue is entirely overrun by mycelial growth. Meanwhile, the glandular elements and the endocuticular tissues are attacked and broken down by the pathogen. Finally, the fungus becomes established in the tracheal muscles and tissues of the gut, whereupon the animal is completely riddled with fungus mycelium. Lefebvre (67) has also reported that mechanical penetration is preceded by enzymatic activity.

That toxins may also be produced has been pointed out by Dresner (38), who attributed paralysis resulting from infection by *B. bassiana* of *Musca domestica* L., *Phythorimoeao perculella* Zeller, and *Gastroidea cyanea* Melsh.,

to a fungus-produced toxin. Sussman (136, 137) also reported that death is probably a result of the accumulation of phenols, which may be produced either by the fungus or by the insect itself.

There are numerous references in the literature to the pathogenicity of *Beauveria* species to various insects. Such work, however, has been based upon the mortality from infection among insect populations sprayed or dusted, in most instances with an unknown quantity of spores, under general laboratory conditions. Sussman (133) has recently mentioned that it may be possible to use the M.L.D. (minimum lethal dose) as a quantitative measure of the virulence of related species of fungi toward various insects. In a later publication (135), he suggested that quantitative studies of virulence, particularly from a genetic or physiological standpoint, could be based on respiratory exchange and weight loss during disease. Because of the absence of a satisfactory quantitative standard of virulence, there is very little information regarding fine variations in virulence that may occur among fungal strains. This is important since, according to Dubos (39), not only does the ability to produce disease vary (at least among bacteria) from strain to strain within the same microbial species, but also within the same strain. It also varies with the state of dissociation of the culture and with its history, both *in vivo* and *in vitro*.

It has been shown very clearly by Westerdijk (148) that the infective power of fungi may be influenced in many ways when different species of parasites are concerned. It is well known that the nutrient elements in culture media have a great influence on preservation or loss of parasitism. Some species of fungi rapidly lose their virulence when propagated on artificial media. Conversely, repeated passage of the fungus through a given host species often results in a marked increase in virulence of this agent for this particular host. It is believed that the M.L.D. of spores of some organisms varies greatly according to the age and vegetative activity of the culture. There is also, as suggested by Dubos (39), the interesting possibility of modifying the optimum environmental conditions under which a given strain is virulent through a process of "training" *in vitro*. This corresponds to the repeated selection of forms within any normal population that are adapted to survival and growth

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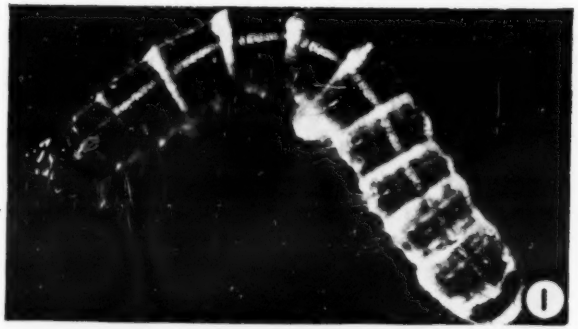
FIG. 1. A mummified larva of *M. americanum* infected with a *Beauveria* strain. Note that the fungus is beginning to protrude from the intersegmental areas. 3 ×.

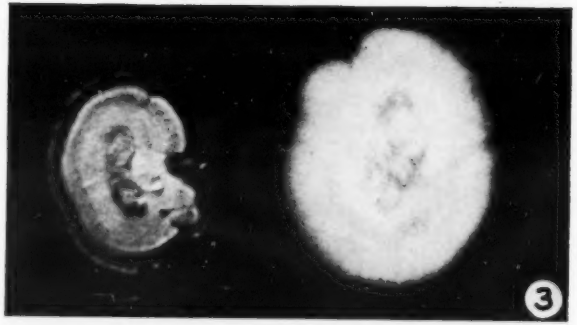
FIG. 2. A number of *Beauveria*-infected *M. disstria* completely enveloped with the vegetative growth of the fungus. The larvae had been surface sterilized in Javex and then placed on Sabouraud maltose agar in a sterilized Petri plate. 1 ×.

FIG. 3. *Beauveria*-infected specimens of *O. autumnata* (on the left) and *E. venata* (on the right). Note the variation in growth between the two larvae. The growth from *E. venata* is much more flocculent. 1 1/2 ×.

FIG. 4. A number of *C. murinana* infected with a *Beauveria* strain. Note the variation in the vegetative growth of the fungus among the different larvae. The specimens were surface sterilized in Javex and placed on artificial medium in a sterilized Petri plate. 1 1/2 ×.

PLATE III





at higher or lower temperatures or humidities. Rigid experimental conditions are of course essential to bring out significant differences in experiments of this nature.

(*b*) *Survey*

In some cases, the material examined (as outlined in materials and methods) from various insect species in this investigation included not more than two or three specimens. In such cases, therefore, it is not possible to make any reference to the importance of the *Beauveria* strains in natural control. In the few instances, however, where large numbers of a particular insect species were examined (Table VI), it was found that the number infected with *Beauveria* among the total number of insects collected varied from 0.5 to 13.1%.

TABLE VI

PERCENTAGE MORTALITY DUE TO *Beauveria* AMONG VARIOUS INSECT SPECIES EXAMINED

Host	Locality	No. of specimens examined		% infected		References
		Larvae	Cocoons	Larvae	Cocoons	
C. pomonella	Kentville, N.S.	—	2931	—	3.3	—
C. fumiferana	Ontario	8216	—	0.5–1.5	—	16, 72, 138, 139
N. sertifer	Strathroy, Ont.	—	10,387	—	7.9	17, 18
P. erichsonii	Saskatchewan Manitoba Ontario	11,179	5030	0.5–1.5	2.8–13.1	52, 53, 72, 73
S. cylindricollis	Brandon, Man.	400*	—	66.3†	—	—

* *400 refers to the number dead, the total number of larvae collected in this instance not being known.*

† *66.3 refers to the percentage of the total dead infected with Beauveria.*

(*c*) *Appearance of Infected Material*

Within the infected material, the fungus had generally formed a hard, compact sclerotium inside the body of the insect, the external chitinous covering being the only part apparently unaffected.

Shortly after the mummified larvae were placed in a sterile, moist environment at room temperature, the fungus began to protrude, at first from the inter-segmental areas (Plate III, Fig. 1) and then all over the body, completely enveloping it with a white coating of mycelium (Plate III, Figs. 2, 3, and 4).

(*d*) *Host Species and Distribution*

In this investigation, only *Beauveria bassiana* strains were isolated; they came from 63 different insect species collected in various localities throughout Canada (Text-fig. 10). The *Beauveria* cultures[1] received from the Province of Alberta were isolated from rodents at the Laboratory of Public Health, University of Alberta. They were found to be identical with cultures isolated from various insect species.

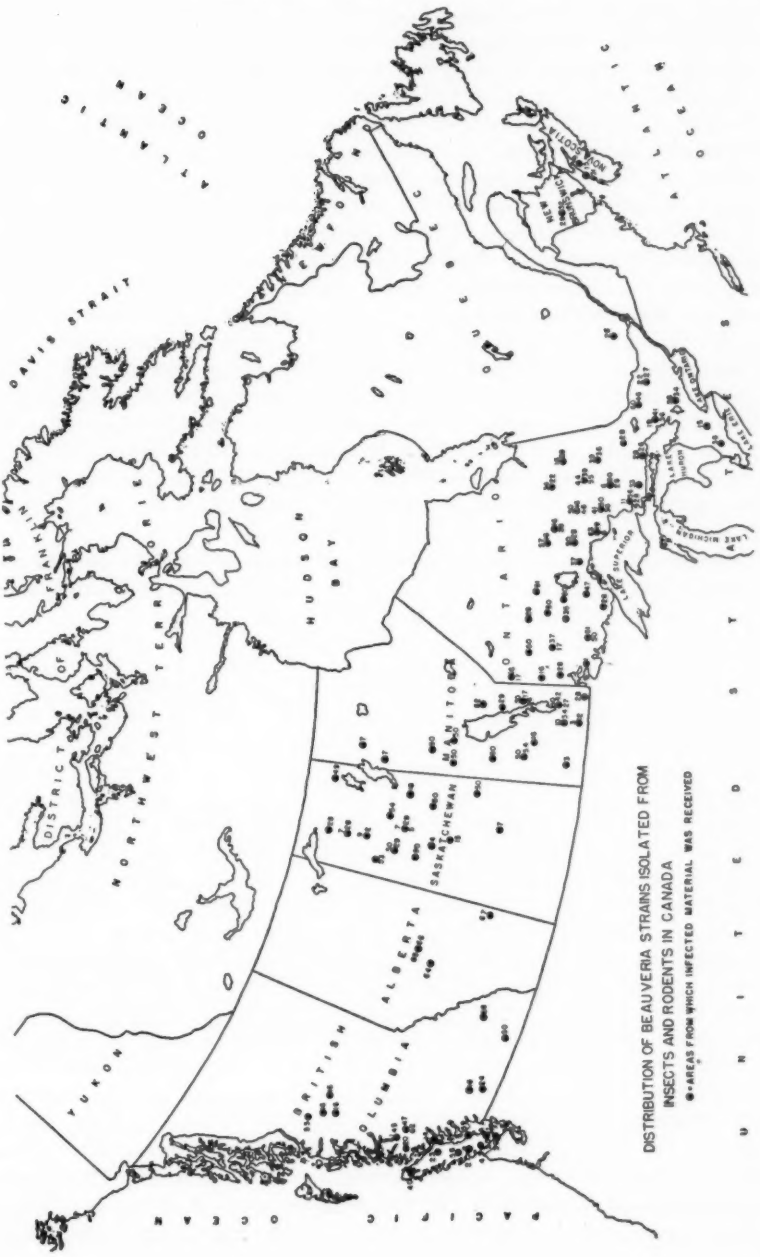
[1] *Data were obtained through personal correspondence with Dr. E. S. Keeping, Medical Mycologist at the Provincial Laboratory of Public Health, University of Alberta, Edmonton, Alberta.*

TEXT-FIG. 10. Distribution of *Beauveria* strains isolated from insects and rodents in Canada. Insect species with which *Beauveria* strains are associated in Canada (see map)

1. *Actias luna* L.
2. *Amphidasis cognataria* Gn.
3. *Anisota virginiensis* Dru.
4. *Anomogyna elimata* Gn.
5. *Anoplonyx occidens* Ross
6. *Archips cerasivorana* (Fitch)
7. " *conflictana* (Wlk.)
8. " *fervidana* Clem.
9. *Arge pectoralis* (Leach)
10. *Autheraea polyphemus* Gram.
11. *Bombyx mori* L.
12. *Brephos* sp.
13. *Camnula pellucida* Scudd.
14. *Campaea perlata* Gn.
15. *Carpocapsa pomonella* (L.)
16. *Choristoneura fumiferana* (Clem.)
17. *Choristoneura pinus* Free.
18. *Cucullis intermedia* Speyer
19. *Diprion hercyniae* (Htg.)
20. *Enypia venata* Grt.
21. *Epicnaptera americana* Harr.
22. Geometridae
23. *Hemichroa crocea* (Four.)
24. *Hyphantria textor* Harr.
25. *Lambdina somniaria* Hlst.
26. Liparidae
27. *Malacosoma americanum* (F.)
28. " *disstria* Hbn.
29. " *lutescens* N. and D.
30. " *pluviale* (Dyar)
31. *Melanoplus* sp.
32. *Mineola vaccinii* (Riley)
33. *Monoctenus juniperinus* MacG.
34. *Neodiprion abietis* (Harr.)
35. " *banksianae* Roh.
36. " *lecontei* (Fitch)
37. " *virginiana* Roh.
38. " *sertifer* (Geoff.)
39. " *swainei* Midd.
40. " *tsugae* Midd.
41. *Nepytia canosaria* Wlk.
42. *Nyctobia limitaria* Wlk.
43. " " *nigroangulata* Stkr.
44. *Nymphalis antiopa* (L.)
45. *Oporinia autumnata* Gn.
46. *Paraclemensia acerifoliella* Fitch
47. Phalaenidae
48. *Pikonema alaskensis* (Roh.)
49. " *dimmockii* (Cress.)
50. *Pristiphora erichsonii* (Htg.)
51. *Sciaphila duplex* Wlshm.
52. *Semiothisa oweni* Swett
53. " *sexmaculata* Pack
54. *Sitona cylindricollis* Fahr.
55. Tenthredinidae
56. *Tolype* sp. poss. *laricis* Fitch
57. Tortricidae
58. *Trichiosoma triangulum* Kby.
59. *Venusia cambrica* Curt.
60. Unidentified insect sp., Ottawa, Ontario
61. Unidentified insect sp., British Columbia
62. Unidentified insect sp., British Columbia
63. *Zenobia pleonectusa* Grt.

Rodents with which *Beauveria* strains were associated in Alberta, for the year 1950 (see map)

64. *Clethrionomys gapperi* (red-backed mouse)
65. *Microtus pennsylvanicus drummondi*
66. *Peromyscus maniculatus borealis* (deer mouse)
67. *Peromyscus maniculatus osgoodi*


DISTRIBUTION OF BEAUVERIA STRAINS ISOLATED FROM
INSECTS AND RODENTS IN CANADA
●=AREAS FROM WHICH INFECTED MATERIAL WAS RECEIVED
DAVIS STRAIT
HUDSON BAY
DISTRICT OF FRANKLIN
NORTHWEST TERRITORIES
YUKON
BRITISH COLUMBIA
ALBERTA
SASKATCHEWAN
MANITOBA
ONTARIO
QUEBEC
NEW BRUNSWICK
NOVA SCOTIA
PRINCE EDWARD ISLAND
NEWFOUNDLAND
LABRADOR
LAKE SUPERIOR
LAKE MICHIGAN
LAKE ONTARIO
ATLANTIC OCEAN
PACIFIC OCEAN
UNITED STATES

Alberta, Prince Edward Island, and Newfoundland are the only provinces from which insect collections were not received. It is believed, however, that *Beauveria* strains are associated with insect species in these areas.

Beauveria strains were also found on material received from various parts of the United States and Europe. The specimens received from the United States include: *C. fumiferana* and a *Crambus* species, Oregon; *E. chalcedona*, Berkeley, California; *P. japonica*, Beltsville, Maryland; *P. nubilalis*, Stoney County, Iowa; and an unknown insect species from Beltsville, Maryland.

The specimens received from Europe include: *C. murinana*, Vosges Mountains of France; *S. impexus*, Rosenheim near Munich, Germany; and *S. rufimitrana*, Prague, Czechoslovakia.

It is not surprising that *Beauveria* strains were found on numerous insect species throughout Canada, parts of Europe, and the United States. It is evident from the literature that members of the genus occur throughout the world. They have been isolated from insects throughout the U.S. (15, 28), Canada (130, 140), England (97), France (40, 102), Germany, Austria, Denmark, Sweden, Norway, Poland, Hungary, Italy (19), Egypt (85), South Africa (119), European and Asiatic Russia (12, 19), China, Japan, Ceylon, Philippine Islands, Manchuria (23, 66), Australia (35), Brazil, Argentina, Uraguay (78), and the Dominican Republic (27).

More detailed references to the host-range and geographical distribution of *Beauveria* species in the United States and in Europe are given in publications by Charles (27) and Gösswald (49).

(*e*) *Control*

It has become evident that, while *B. bassiana* and other species have often proved to be costly to the sericultural industry, they are, nevertheless, of some importance in the natural control of many noxious insects.

Considerable numbers of *C. pomonella* were found infected with *B. bassiana* in some New Jersey apple orchards (59). Laboratory tests showed the fungus to be highly pathogenic when spores were either dusted upon, or inoculated into, healthy, hibernating or freshly spun-up larvae. In the field, artificial dissemination of spores on the foliage brought about a significant increase in mortality. The fungus appears to be particularly important in cool, wet seasons. Another *B. bassiana* strain has been reported (43) to be pathogenic to the codling moth, even under relatively dry conditions (40% R.H.) at Chamusca, Portugal. *B. bassiana* has been reported (4) as the only disease organism in the United States that has been observed to kill *P. nubilalis* larvae in the field. In this case, however, the mortality was directly traceable to artificially imposed conditions. A mortality of 60–70% was obtained in field tests in Ontario (8, 130) in which corn borer larvae were dusted with *B. bassiana* spores.

More than 30% of the females of *Diabrotica undecimpunctata* Mann., collected in the early spring, are sometimes killed within a period of two weeks by the *globulifera* strain (112). In tests with the coconut leaf miner, *Promecotheca cumingi*, this fungus has caused a mortality ranging from 44 to 67%

(109). It is also known to have caused considerable loss among populations of the Philippine migratory locust, *Pachytylus migratorioides* (108), and is regarded as the most destructive natural enemy of the chinch bug, *Blissus leucopterus* (15, 51, 89, 121, 125, 126).

Although "*B. effusa*" and "*B. doryphorae*" are known to kill up to 50% of enfeebled and hibernating Colorado potato beetles, *Leptinoptarsa decemlineata* (Say), in France, Belgium, and other potato growing areas, they are unable, even under favorable conditions, to control the vast underground populations of this insect (20).

Despite the fact that "*B. densa*" has caused epidemics under natural conditions among larval populations of the cockchafer in various parts of Germany, Blunck (19) has expressed doubt regarding its potentialities for artificial control on any considerable scale. According to Karpinski (61), dusting of woods infested by *Melolontha* sp. with a 4 : 1 talc–spore mixture at a rate of 40–50 kgm. per hectare gave an incidence of infection of 44.6% at the center falling to 24.5, 18.2, 11.2, 5.8, 4.8, and 3.7% within a radius of 1, 2, 3, 4, 5, and 6 km. respectively.

Many other investigators (2, 24, 37, 50, 54, 60, 66, 79, 80, 93, 94, and 141) have referred to the value of *Beauveria* "species" in the natural control of insect pests.

Investigations in this laboratory on the artificial dissemination of *B. bassiana* spores on populations of *P. erichsonii* were conducted during the summer months of 1950 and 1951 mainly for the purpose of studying methods and procedures in the field. Detailed reports regarding techniques and results have been presented in the Annual Technical Report, Forest Insect Laboratory, Sault Ste. Marie, by MacLeod (72) and by Heimpel (52, 53). It was found that the dissemination of *Beauveria* spores on 5332 larvae of *P. erichsonii* under field conditions resulted in a mortality ranging from 2.9 to 9.5%. Examination of 3063 cocoons from larvae that had been sprayed or had spun up in ground previously sprayed showed a mortality due to *Beauveria* infection of from 2.5 to 30.8%. In non-sprayed control areas, the mortality among larvae ranged from 0.5 to 1.5%, and among cocoons from 2.8 to 13.1% (Table VI).

The increased mortality following spraying is attributable in part to the fact that some of the sprayed larvae were reared in small containers under laboratory conditions. The environment was then more conducive to the germination and development of spores; hence the larvae were less apt to escape infection than were those reared under natural conditions (72).

2. ON ANIMALS

Smith (124) reported that she had isolated *B. tenella* from the flesh of dead birds.

Kuru (62) found that "*B. shiotae*" showed a certain pathogenicity for guinea pigs and rats when injected into the abdominal cavity, he also reported that Majoki found it pathogenic to silkworm larvae.

Dresner (38) reported that slugs (*Limax* sp.) dusted in a moist chamber were killed by *B. bassiana* spores. He also found that no adverse effects developed when two rats (*Rattus norvegicus* L.) were fed a suspension of 0.5 gm. of *B. bassiana* spores mixed with bran and molasses. Similarly no adverse after-effects developed when 0.25 cc. and 0.5 cc. of a 1% suspension were injected into the body cavity and right shoulder muscle, respectively, of a rat.

According to Dr. E. S. Keeping (personal communication) *Beauveria* strains were found within the lung tissues of 14 rodents collected by the Laboratory of Public Health in Alberta in 1950. So far histological examination has not shown that the fungus creates a pathological condition within the tissues.

Aschieri (1) found that a subcutaneous inoculation of "*B. epigea*" produced an ulceration, which gradually healed with the formation of a cicatrix, in white mice. This apparent recovery did not last long, however, for the animal died within a few days after cicatrization of the wound. An autopsy showed the presence of pulmonary lesions. Aschieri concluded that he was dealing not with a pathogenic organism but rather with a saprophyte that at times can bring about pathological changes. This phenomenon may be due to the fact that the organism gradually becomes adapted to parasitic life, and in addition there may be a diminished resistance on the part of the host organism.

3. ON HUMANS

"*Beauveria epigea*" was isolated from cutaneous and osseous lesions on the feet of a patient in Venice (1).

"*Beauveria shiotae*" was isolated from an abscess in the right hypochondrium of a patient in Japan (62). Owing to the sudden death of the patient, however, it was not possible to determine the role of the fungus with certainty, although it was concluded that cholemia was the probable cause of death.

In handling sporulated material of *B. bassiana*, Dresner (37) reported that small clouds of dust (spores) were often inhaled. They had no harmful effect on the operator.

An interesting reference, cited by Dieuzeide (34), reports that *Beauveria* spores ground into a fine powder and taken with a little water and tea is an excellent remedy for infantile meningitis and epilepsy.

4. ON PLANTS

Beauveria bassiana spores inoculated under controlled conditions into San Marzano tomato fruits produced an ordinary rot indistinguishable from that induced by various plant pathogens (28).

When *B. bassiana* spores were placed on bean plants, it was noted that some of the spores germinated and sporulated on the hairs of the underside of the leaf, but with no serious effects. The fungus was able to germinate and grow when sprayed or dusted on leaves removed from the stem and kept in a moist chamber. A liquefaction of the plant tissue in the area of growth was noted

(37). No harmful results occurred when spores (0.5%) were dusted on an orange fruit (*Citrus sinensis* Osbeck). Grasses and weeds on which insects had died were not affected by the fungus although the humidity was optimal for germination (37).

"*Beauveria globulifera*" according to Steinhaus (131) grows on corn and certain other plants but not as well as on insects. Its spores, sprayed on the leaves and growing points of coco seedlings, had no ill effects. Reyes (109), therefore, concluded that this fungus could be used extensively for insect control purposes, without fear of damaging the coco plants except perhaps through mechanical injuries or when it is transported through wounds or tunnels made by insects.

5. AS SAPROPHYTES

Siemaszko (122), in referring to the saprophytic development of members of the genus *Beauveria* on the remains of plants, concluded that it must be caused by the presence of the remains of insects that contain sclerotia or mycelium.

B. MEMBERS OF THE GENUS *Tritirachium*

With some *Tritirachium* species, there is believed to be varying degrees of pathogenicity, although for the most part they appear to be saprophytic in nature.

Tritirachium cinnamomeum Beyma (14) was isolated from a fly found in the cretaceous caves of Maastricht (Netherlands).

Tritirachium musae Beyma (13) came from a banana leaf spot. Beyma (13, 14) does not give the source of the fungus *T. purpureum* (Saito) Beyma, but has concluded that it is almost identical with *T. musae*.

Tritirachium oryzae Vincens (143) was isolated from sclerotia of the fungus *Sclerotium oryzae* and is believed to be capable of harming rice seedlings by producing a decay of the young roots. Vincens found that grains of rice that had hardly begun to sprout when placed in Petri plates containing pure cultures of *T. oryzae* showed disease symptoms within six days. At the end of that time, the seedlings not in contact with the mycelium of the fungus were several centimeters high, green in color, and well rooted, whereas those that were in contact with the fungus were short and their roots brown, and in some cases the plants were withered and practically liquefied, almost to the base where the rootlets began to appear.

Vincens (143) also observed the presence of this fungus on very young leaves of rice plants collected near Mytho that were infected with a disease designated by the natives as "Boyau de Canard", a disease characterized by the withering aspect of the young leaves.

Tritirachium brumpti Langeron (64) was obtained from a case of Kerato-conjunctivitis in a 17-year-old European girl resident in Cairo. Maran (74) conducted pathogenicity tests with this species (obtained from C.B.S.) against several species of insects. He found that *Tenebrio molitor*, *Calandra*

granaria, and *Ephestia kuhniella* were susceptible to infection, while *Phagiodera versicolora*, *Melasoma aenea*, *Forficula auricularia*, and *Aleurobius farinae* appeared to be unaffected. He found that the greatest pathogenicity was obtained with spores removed from cultures grown on artificial media in which the basic medium (beer wort) was supplemented with a decoction from insects.

Tritirachium roseum Beyma (14) was isolated from litter in a cow stable at the Prussian Experimental Station for dairy farming at Kiel.

Tritirachium spicatum (Ferraris) Limber was, according to Limber (68) isolated by Ferraris (42) from the underside of a cork used as a stopper in a flask of picric acid.

Tritirachium dependens Limber (68) came from the dead roots of the plant *Yucca treculeana.*

Tritirachium album Limber (68) is believed to have been discovered with the species *Penicillium intricatum* from a book in the basement of the Marine Biological Laboratory, Woods Hole, Mass. *T. heimii* Saccas (117) was likewise isolated from a book at the Public Library, Thiveral, and also from a packing-box in a cellar at Juan Gulf, France.

Langeron (65) concluded that, medically, *Tritirachium* species appear to be cosmopolitan saprophytes, accidentally attacking man. Their pathogenic powers up to the present seem to be extremely weak.

Tritirachium species have been encountered in the Netherlands, Japan, Antilla, Egypt, Germany, the United States, France, and Canada.

C. Discussion

In nature representatives of the genus *Beauveria* are a continuous factor in the natural control of many insect pests. It appears, however, that the mortality caused by this fungus is not as great as is suggested in publications. In many cases the high mortality reported is believed to be due to the rearing of field-collected material in the laboratory. Under such conditions insects are much more subject to *Beauveria* infection than when in their natural environment. It has been found that a heavy kill generally develops within a period of 6 to 10 days when spores of *B. bassiana* are sprayed or dusted on larvae of *P. erichsonii*, *C. fumiferana*, *C. pomonella*, *M. disstria*, and *D. hercyniae* under laboratory conditions. Under field conditions, however, the artificial dissemination of spores on feeding *P. erichsonii* larvae failed to bring about a marked increase in the incidence of *Beauveria* infection. More promising results followed ground-spraying just before the larval population dropped to the ground to form cocoons.

While *Beauveria* species develop primarily on insects, isolated cases have been reported where strains were isolated from humans, guinea pigs, and rats; their role in each case was, for the most part, uncertain. They apparently do not attack plants. The fact that the conditions that determine the occurrence and extent of the disease are not well known emphasizes the necessity for more complete ecological and physiological studies of the organisms and their host-insects.

There are one or two instances where members of the genus *Tritirachium* have been reported on humans, plants, and insects. They exhibit very weak pathogenic power and for the most part are regarded as saprophytes with a world-wide distribution.

Summary and Conclusions

Beauveria bassiana was isolated from dead insect material more frequently than any other type of entomogenous fungi. This species was, therefore, selected for investigational work concerning the problems of utilizing entomogenous fungi in the biological control of insect pests.

The investigation developed into an essentially morphological and taxonomic study. It is obvious that insect pathologists must know the correct names and synonymy of the fungi with which they are working; otherwise results cannot be correctly interpreted by others and may lead to serious misunderstanding.

In an account of the genus *Beauveria*, Petch (95) pointed out that European writers refer to this genus as *Botrytis*, while American mycologists call it *Sporotrichum*. In view of this confusion, Vuillemin (145) instituted the new generic name *Beauveria*, which he dedicated to Beauverie, since the latter (9) had forseen the necessity for creating a new genus to accommodate *Botrytis bassiana*, the agent of muscardine of the silkworm. Vuillemin's description of the genus was based essentially on the mode of spore development.

Since then various investigators have added further components to the genus, so that there are now a large number of species credited to it. The descriptions of most of these are based on such slight biological differences as to render them of doubtful validity. To adhere to the set pattern would eventually result in each insect species having its own particular species of *Beauveria*. Moreover, the fact that insects are liable to attack by more than one type of isolate, as is shown in the present investigation, immediately suggests the prospect of an illimitable series of species.

A critical analysis of the characteristics (rate of growth, amount of aerial mycelium, formation of coremia, amount of sporulation, and color on the surface and in the substrate) adopted by earlier investigators to differentiate between alleged *Beauveria* species has shown that they are not sufficiently outstanding or prominent as criteria upon which to establish species. Moreover, these characteristics are unstable and can be changed simply through monospore culturing or by transferring cultures from one type of medium to another. It is therefore concluded that only two species, viz. *B. bassiana* and *B. tenella*, exist among the numerous cultures studied. Between these the only apparent difference is that *B. bassiana* has *ca.* 50% globose spores and *B. tenella*, *ca.* 98% oval spores. When the extent of variability in these species had been determined, their former descriptions were accordingly modified. A variability study of *Tritirachium* species has not been completed. An examination of some cultures, as well as a comparative study of species

descriptions, indicates that some species can be reduced to synonymy. In general, their mode of spore development appears to be identical with that of *Beauveria*. This similarity led Saccas (117) to conclude that *Tritirachium* species should be transferred to *Beauveria*. It is evident, however, from the illustrations and discussion in this paper that morphological and biological differences exist between them. *Tritirachium* should not, therefore, be treated as a synonym of *Beauveria*.

It was finally concluded from a comparative study of the literature on the so-called *Beauveria* and *Tritirachium* species that 14 were reducible to synonymy with *B. bassiana* and *B. tenella*; 10 belonged to the form genus *Tritirachium*; one, "*Tritirachium epigaeum*", could not, on the basis of published accounts, be placed with certainty in either genus; and seven were excluded from both.

From a review of the literature it is evident that members of the genus *Beauveria* are primarily parasitic on insects, while members of the genus *Tritirachium* are primarily saprophytes.

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CORRECTIONS

Page 123. At the upper left of the page, above "Apotheciis erumpentibus—", should appear the heading: **Durandiella alni** sp. nov. (Figs. 8, 21, 32).

Page 434. In the illustration (Plate I) that faces this page, the correct magnifications are: × 575 for Figs. 1, 3, 5, and 7; × 1150 for Figs. 2, 4, 6, and 8.

Page 436. Line 23 should read "confluent and thus elongate, not crowded, 1–2.5 μ wide × 1–6 (10) μ."

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